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A Quarterly of Research

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THE NORTHERN COPPERHEAD IN IOWA[1]

REEVE M. BAILEY

Agkistrodon contortrix was listed among the reptiles of Iowa (Somes, 1911:150), but subsequent authors, lacking confirmatory evidence, have not included the state as being within the range of the copperhead.

Four specimens now furnish an authentic basis for extending the known range of the northern copperhead, *Agkistrodon mokasen cupreus* (Rafinesque)[2], to include southeastern Iowa. These individuals were taken from the wooded, rocky bluffs of the Mississippi and lower Des Moines rivers, terrain typical of the habitat preference elsewhere. The records are as follows: (A)—LEE Co., vicinity of Keokuk,, Iowa State College collection, about 1895, C. F. Davis. (B)—LEE Co., bluff of Mississippi River, 3.5 miles north of Keokuk, Chicago Academy of Sciences collection, September, 1939, R. F. Trump. (C)—VAN BUREN Co., Bonaparte, I. S. C., August, 1939, R. F. Trump. (D)—VAN BUREN Co., Bonaparte, I. S. C., July 28, 1939, R. F. Trump.

These snakes agree well with Gloyd and Conant's description (1938: 164-165). In each there are 8 upper labials; specimen A has 9 lower labials, the others 10. Specimen C has 2 preoculars and 3 postoculars, the others have 3 preoculars and 2 postoculars. The more or less spool-shaped crossbands involve from 2 to 3 scales in a lengthwise series along the mid-dorsal line and from 5 to 11.5 along the widest part on the sides; these average (A) 7-3-7; (B) 8-2.5-8; (C) 7-2.5-8; (D) 10-2.5-10. Other features of scutellation and pattern are given in the accompanying table.

Spec.	Sex	Ventrals	Caudals	Total Length (mm.)	Tail length (mm.)	Body blotches	Scale rows
A	♂	152	48 (15 divided)	801	103	17	25, 23, 21, 19
B	♂	153	47 (5 divided)	585	86	14	25, 23, 21
C	♂	149	47 (12 divided)	790	104	16	25, 23, 21, 20
D	♀	150	44 (12 divided)	775	95	15	25, 23, 21, 20

[1]

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        1938. The subspecies of the copperhead, *Agkistrodon mokasen* Beauvois.
            Bull. Chicago Acad. Sci., 5(7):163-166, figs. 1-3.

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AGE CLASSES OF WINTER COVER USED BY THE EASTERN BOB-WHITE, COLINUS V. VIRGINIANUS, IN SOUTHERN IOWA[1,2]

Maurice F. Baker

Woody growth along gullies forms some of the most favorable winter cover for the eastern bob-white *(Colinus v. virginianus)* in southern Iowa and similar localities. Workers advocating gully plantings for erosion control and as a game management practice are sometimes asked how old a planting must be to attract and hold quail during the winter, or they may be asked at what age timber may be thinned for lumber or fuel, and still leave good bob-white cover.

Errington and Hammerstrom (1936) concluded that a given territory has a given year to year carrying capacity for quail, barring catastrophic events which would decimate the population, and provided that no significant change in cover conditions occurred, but did not analyse in detail the character of the cover used. Green and Beed (1937) attributed much of the 35 per cent decrease in the quail population that occurred during the abnormally severe winter of 1935-1936 to cold and hunger after much of the winter cover had been filled with snow, and pointed out that during periods of heavy snow and drifting the quail sought out and used gully coverts extensively.

In an effort to learn something of the age classes of winter cover used by quail, especially that along gullies, a study of such coverts and their usefulness to quail was made during the winter of 1937-1938 and 1938-1939 in Davis County, Iowa. This investigation was under the supervision of Dr. G. O. Hendrickson, Assistant Professor of Zoology, Iowa State College; Dr. Logan J. Bennett, Associate Biologist of the United States Biological Survey, and his successor, Thomas G. Scott.

The terrain of Davis County, Iowa, is typical of much of southeast Iowa. It is rolling to rough, and has many wooded draws and gullies extending into the cultivated fields. A series of various aged coverts along gullies was picked at random from approximately the central two-thirds of the county. Early in the course of the investigation it became evident that a covert, for study purposes, must be relatively isolated from other cover, and have an adequate food supply adjacent. Thus, all coverts kept under observation had a cornfield adjacent to them, and were not connected with other cover by good travel lanes.

Determination of the age of the coverts was facilitated by the use of a wood-increment borer. Several representative trees were examined

in each covert, and the average age of the sample trees taken as the age of the covert. Study areas were mapped accurately so that the size of the coverts could be determined with a planimeter. A comparison of the size of the coverts in relation to the use by quail indicated that several of those originally selected may have been too small to attract quail in winter. All such coverts were dropped from consideration in the age series.

Censusing the bob-whites was accomplished by flush counts, but track counts and other signs were used to supplement this method. Dust baths, night roosts, loafing spots and scattered fecal droppings and feathers all were of more or less value in indicating the presence of quail. No dog was used during the first winter, but during the second a pointer dog was used to good advantage in locating the quail. Some influx and egress to and from the study areas occurred, especially during the second winter, but generally the population of a given area became quite stable with the advent of cold weather.

The survival for each age class is presented in table 1. All pertinent data are given in the table and a short discussion of the data follows the table. The fall count was made after the covey had assembled for the winter, and the last spring count was made early in March before the spring dispersal, with counts about once each week during the winter. A blank space in a population column indicates that the covert was not kept under observation during that season. A question mark indicates that the population for that season was not determined, but that sufficient data were obtained later in winter to justify the inclusion of the area in the scope of the investigation. This applies on a few areas not kept under regular observation during the second winter.

TABLE 1 *Bob-white populations in the several age classes of winter cover*

Area number	Population				Remarks
	Fall 1937	Spring 1938	Fall 1938	Spring 1939	
1- to 10-year age class					
1			0	14	Food conditions highly satisfactory during the winter
2	14	15	?	14	Food conditions fair both seasons
3	0	0	0	26	Food conditions about the same both seasons
11- to 20-year age class					
4			16	15	This area had as many as 20 quail during part of winter
5			15	10	Loss from egress during early winter
6	0	0	17	14	No corn adjacent to covert during second winter
7	17	0			Loss during first year from egress

TABLE 1. (continued)

Area number	Population				Remarks
	Fall 1937	Spring 1938	Fall 1938	Spring 1939	
8	10	10	18	21	Food conditions only fair both years
9			12	24	Covert large and branched; food fair; heavy influx
10			0	0	Large covey during most of winter; food scarce
11			14	14	Another covey present occasionally; food scarce
12			16	16	Food only fair; cover mostly of willows
13	14	10	17	17	1938 loss probably due to late winter egress

21- to 30-year age class

Area number	Fall 1937	Spring 1938	Fall 1938	Spring 1939	Remarks
14	18	18	17	38	Influx late in the winter of 1939
15	10	10	11	10	Food conditions rather poor both years, loss from predation
16			0	0	7 quail during most of the winter, predation of one
17	0	0	13	3	Heavily grazed each winter by cattle and sheep
18			13	10	Food fair, cause of loss undetermined
19	12	12	16	16	Another covey present occasionally second winter
20	0	0	17	13	Corn shocked and removed each year, cause of loss unknown
21	15	0	?	9	No corn during second winter, egress during first winter
22	20	15	20	20	No corn second year
23	0	15	0	0	Food conditions good first year and poor second year
24			22	22	Food conditions good, covert not very dense
25			26	10	Food conditions very poor, loss from egress

31- to 40-year age class

Area number	Fall 1937	Spring 1938	Fall 1938	Spring 1939	Remarks
26			20	16	Cause of loss undetermined, food fair
27			20	11	Egress of all but 13 in the fall, predation of two
28			13	16	Food conditions in the cornfield poor
29	13	9			Loss due to hunting in November, 1937
30	11	16	15	15	Two other coveys present occasionally during second year
31			14	19	Loss of one quail by predation after late winter influx

TABLE 1. (continued)

Area number	Population				Remarks
	Fall 1937	Spring 1938	Fall 1938	Spring 1939	
41- to 50-year age class					
32			13	13	Food conditions good, population stable all winter
33	13	15	20	18	Quail shifted considerably during winter
34			13	9	Interspersion with feeding grounds rather poor
35			16	16	Two other coveys present occasionally, food conditions good
36	0	0	30	30	No corn adjacent to covert second year, but natural foods abundant
37			22	22	Covert rather small, food conditions only fair
38			0	0	Food conditions only fair, covert poorly isolated
39			22	22	Another covey occasionally present
40			12	12	40 quail present at times, loss of one by predation
51- to 60-year age class					
41			17	11	One quail lost by predation, egress of five
42			6	4	Food conditions good, loss of two by predation
43			40	17	11 other quail present occasionally, some hunting
44	15	15	18	18	Covert pastured, food only in fair abundance each year
45			0	0	Food conditions good, quail present part of time
46			14	14	Influx of ten quail in March not included in figures
47			17	17	A few other quail occasionally present
48			40	19	Egress of 20 during fall, loss of one by predation
61- to 91-year age class					
40			15	24	Influx late in winter, food conditions only fair
50	17	17	30	12	Food conditions good, no apparent loss from predation
51			35	27	20 other quail present at one time

The cover in each of Areas 1, 2, and 3 (table 1) was made up primarily of sprouts that had grown up around the stumps of former trees, and tall coarse weeds. The large covey at Area 3 during the second winter may not have been entirely dependent on that cover, but appeared to spend most of their time on the area.

It is evident that at Area 6 there was a good survival the second winter and none the first winter, although the food conditions did not appear as favorable during the second winter. Area 10 held a fair-sized covey during most of the winter, but the surrounding fields were heavily grazed, and the quail seemed to move into an adjacent territory where there was an abundant weed growth in a small grain stubble field.

The 21- to 30-year age class is quite representative of the two year's survival data. Survival in this age class for the second year was greater than or equal to that of all coverts except one that were kept under observation both years. This tendency is not so apparent in all age classes, but is more apparent when all the study coverts are considered. Area 23 is one of the few in the entire series that did not hold more quail during the second year. The covey present the first winter came into the area rather late in the season. During the second winter a large covey fed in a cornfield bordering the covert, which these birds were not known to use.

At Area 27 all the adjacent corn was shocked, and the available feeding range was rather small. Area 31 carried 14 quail without loss during most of the winter. Shortly after six more quail had come into the area late in February, one was taken by a Cooper's hawk *(Accipiter cooperi)*.

The covert at Area 34 is not of uniform age throughout. Part of it is about 25 years old, and is more dense than the older portion. The quail abandoned the older portion for the more dense portion during the winter, even though it was adjacent to the cornfield.

Area 38 did not serve regularly as quail cover, but was used in conjunction with other cover by 14 quail.

At Area 42, predation occurred during the winter. This area was part of a concentration area for about 15 ring-necked pheasants *(Phasianus colchicus torquatus* (Gmelin), which, with the quail, made up a heavy game population in the vicinity. Area 43 is part of an organized game management area and probably is hunted over more than any other one in the series. Area 45 was used to a considerable extent throughout the winter by a covey of quail. However, these birds also used some nearby river bottom cover, and for that reason are not counted.

All of the 61- to 90-year coverts were large enough to permit the quail to shift their feeding ground during the winter without having to leave the covert. This occurred at each of these Areas 49-51. The loss of birds from Area 50 occurred late in the winter.

During the two winters when these observations were carried on the weather was relatively mild and favorable to the survival of quail. This similarity is fortunate in that it eliminates the variable of winter weather from the comparison of the two years' data.

The months of November and December of 1937 were 3.6° F., and

1.3° F. below normal. January and February of 1938 averaged 3.7° F.
and 8.8° F. above the normal temperatures. Precipitation for the same
period was 0.25 inches above normal with 7.4 inches of snow distributed
evenly over the winter period.

During the same months of the winter of 1938-1939 the departure
from normal temperature was 2.0° F., 2.1° F., 10.8° F. and 0.0° F. above
normal, with a minimum of 5° F. below zero on February 10. Precipitation
for the same period was 1.3 inches above normal with a total snowfall of
13.7 inches. Some snow fell each month, with the greatest amount occur-
ring on February 28, when 6.5 inches fell.

The covert of Area 23 was of the average age for 1937-1938 and of
Area 27 of average age for the year 1938-1939. It is evident that the indi-
vidual coverts which were kept under observation during both winters
of the investigation generally had more quail surviving the second winter
than had survived the first winter. Errington and Hammerstrom (1936)
have shown that a given territory has a certain year to year carrying
capacity for quail. Therefore, it is reasonable to believe that these coverts
were not, as a rule, filled to capacity with quail during the first year.
Similarly it is not unlikely that since most of the coverts were not filled
to capacity during the first winter that the county as a whole was not
populated to capacity. If these suppositions are true the absence of quail
at certain apparently favorable coverts during the first winter can be
explained. At coverts on Areas 3, 6, 17, 20, 21 and 36 at least a few quail
survived the second winter, as none was found the first winter. Areas
6, 20, 21 and 36 had food conditions less favorable during the second win-
ter. To the writer these facts suggest that a much heavier population of
quail prevailed in Davis County during the second winter of the investi-
gation, with a subsequent filling up of a greater number of the favor-
able coverts.

It is interesting to note that on Areas 25 and 36 quail were known to
have been hatched and reared near these coverts during the summer of
1938. At Area 36 approximately the same number of quail was noted
during the summer as remained during the winter. These facts suggest
at times of low population density the presence or absence of quail at a
given covert during the winter may not be determined by the favorable-
ness of cover as much as by quail reared in the immediate vicinity.

During the first year of the investigation 20 coverts were kept under
observation. Of these, seven, or 35 per cent, were vacant at the close of
the season. The average age of the coverts was between 25 and 26 years.
Fourteen of the 20 coverts were below the average age, and of these, six,
or 42.8 per cent, were vacant. Of the older group of coverts, one, or 16.6
per cent, was vacant. Expressed in another way it may be said that six
of the seven, or 85.7 per cent, of the vacant ones occurred in those below
the average age class.

During the second winter 49 coverts were kept under observation.
Five, or 10.2 per cent, of these were vacant during the late winter. Those
below the average age class, three of 26, or 11.5 per cent, were vacant,

while but 2 out of 23, or 8.7 per cent, were vacant in the older group. Sixty per cent of the vacancies occurred in the younger age class group.

Table 2 presents a summary of these various figures. All the coverts observed the first year are added to those that were observed the second year giving a total of 69 coverts. All percentages are figured on a basis of these 69 coverts giving average figures for both years' observation.

TABLE 2. *Summary of survival by age classes*

	Number of coverts	Percentage vacant	Percentage of vacancies	Average quail to unoccupied covert
Coverts below average age ..	39	23.2	75	11.2
Coverts above average age ..	30	10.0	25	14.8

From table 2 it is apparent that not only did the greater percentage of vacant coverts occur in the younger group, but also that the older coverts had an average carrying capacity greater than the younger group.

SUMMARY

1. The research, concerned with the age classes of winter cover suitable to quail, was carried on over a two-year period in Davis County, Iowa.
2. Weather conditions were similar and slightly above normal during the two years.
3. Population of quail in the county as a whole appeared to be somewhat higher during the second winter. Sixty-five per cent of the observed coverts were occupied the first winter, and 89.8 per cent were occupied the second winter.
4. Variables such as food and roosting cover were reduced to a minimum by selecting for observation coverts which were adjacent to cornfields, but agronomic practices during the fall and winter produced unpredictable variations in these factors.
5. From the data obtained it appeared that any age class of covert might be adequate for quail in winter if other factors are favorable, but that those coverts above the 24-28 year age class have a definitely greater use for quail. Over the two year period 75 per cent of the vacant coverts occurred in coverts below the average age class. The average number of quail in the younger group was 11.2 and the older group 14.8.

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   1937 Climatological data, Iowa Section, in cooperation with the Iowa Dept. of Agriculture, 48 (11 and 12).

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   ———— 1939. Climatological data, Iowa Section, in cooperation with the Iowa Dept. of Agriculture, 50 (1 and 2).

PLATE I.

Fig. 1. Area 1. Cover composed principally of sprouts and tall weeds.
Fig. 2. Area 19. Cover at extreme right is somewhat older than the remainder.
Fig. 3. Area 36. Ungrazed, normally developed cover, along a deep gully.
Fig. 4. Area 44. Lightly grazed cover with good undergrowth.

PLATE I.

Fig. 1

Fig. 2
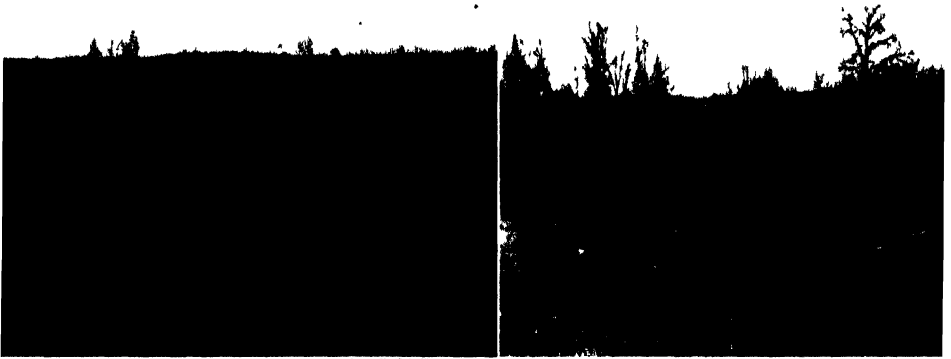
Fig. 3
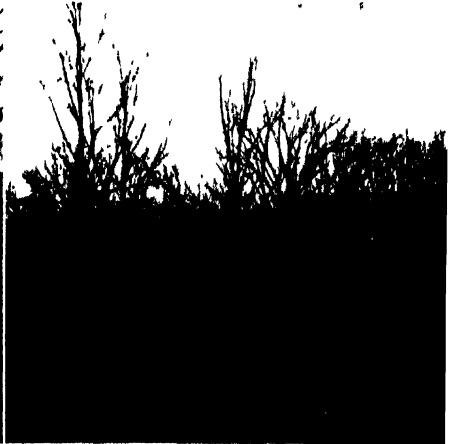
Fig. 4

HIGH-MOLECULAR-WEIGHT ALIPHATIC AMINES AND THEIR DERIVATIVES[1]

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From the Department of Chemistry, Iowa State College

With the recent trend in organic chemistry toward aliphatic chemistry, a larger number of entries is to be found in the field of high-molecular-weight aliphatic amines. This increase results from the fact that these amines and their derivatives are finding wide application as emulsifiers[2], waxes[3], disinfectants and preservatives[4], detergents[5], textile acids[6], components of drying oils[7] and color lakes[8]. Also, this class of compounds has recently increased in importance since the production of fiber-forming polyamides ("Nylon") involves the use of aliphatic amines as building blocks[9]. Further, the chemistry of high-molecular-weight · aliphatic amines has recently attained a new importance in the isolation and determination of structure of natural products. Researches on the lichen substances[10] and plant waxes[11] have shown that high-molecular-weight aliphatic amines are isolated in degradation studies; consequently, this information makes it important that adequate derivatives exist for the identification of the amines.

DISCUSSION

The pioneer work of Ralston and co-workers[12] on the direct ammonolysis of high-molecular-weight aliphatic acids to nitriles was simplified by McCorkle[13]. These investigations led to further simplification of the apparatus with no loss in yields; in fact there was a slight gain. Moreover, the direct ammonolysis of aliphatic acids was extended to dibasic aliphatic acids with the preparation of sebaconitrile from sebacic acid.

n-Octadecylamine was prepared by the procedure of Adam and Dyer[14]. The use of sodium and ethanol as a reducing agent was found to

[1] Taken in part from a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry, July, 1940. Doctoral thesis number 590.

[2] U. S. patent, 2,146,280 (1939) [*C. A.* 33, 3495 (1935)].

[3] English patent, 507,244 (1939) [*C. A.* 34, 557 (1940)].

[4] Swiss patent, 192,997 (1937) [*Chem. Zentr.*, I, 3799 (1938)].

[5] French patent, 780,044 (1935) [*ibid.*, II, 3723 (1935)].

[6] German patent, 650,664 (1937) [*ibid.*, I, 1497 (1938)].

[7] French patent, 814,698 (1937) [*ibid.*, II, 3241 (1937)].

[8] English patent, 460,147 (1937) [*ibid.*, I, 5060 (1937)].

[9] U. S. patent, 2,130,947 (1938) [*C. A.* 32, 9497 (1938)].

[10] Asahina, Y., "Fortschritte der Chemie Organischer Naturstoffe," Springer, Vienna (1939), Vol. II, p. 27.

[11] Blount, Chibnall and Mangouri, *Biochem. J.*, 31, 1375 (1937).

[12] Ralston, Harwood and Pool, *J. Am. Chem. Soc.*, 59, 986 (1937).

[13] McCorkle, Doctoral Thesis, Reactions of High-Molecular-Weight Acid Derivatives, Library, Iowa State Collee (1938).

[14] Adam and Dyer, *J. Chem. Soc.*, 127, 70, (1925).

involve no danger, since the reaction progressed moderately even at a reflux temperature. In fact, it can be recommended as superior to the use of sodium and n-butyl alcohol[15]. The catalytic preparation of high-molecular-weight aliphatic primary amines[16] was extended to aliphatic diamines with the preparation of 1,10-decanediamine.

High-molecular-weight aliphatic primary amines rapidly absorb carbon dioxide and moisture from the air to form the amine carbamate[16]. n-Dodecylammonium N-n-dodecylcarbamate was prepared from these reagents. It is essential, therefore, both in the preparation and reactions of these compounds, to avoid these contaminants.

High-molecular-weight aliphatic primary amines were found to give urea derivatives with ease. The reaction of n-dodecylamine with carbon disulfide[17], phenyl isothiocyanate and α-naphthyl isocyanate required no special techniques, and gave corresponding thiourea and urea derivatives in compensating yields. Similarly, the reaction of n-octadecylamine with potassium cyanate[17], carbon disulfide and α-naphthyl isocyanate proceeded with no difficulty. The success of the reactions of n-dodecyl- and n-octadecylamines with α-naphthyl isocyanate was probably a result of the precautions taken to dry the apparatus[18].

While high-molecular-weight aliphatic primary amines gave urea and thiourea derivatives with no difficulty, the secondary amines, as a result of their lower relative reactivity, required special techniques. Di-n-octadecylamine did not react at all with carbon disulfide to give the expected tetra-substituted thiourea. It reacted with potassium cyanate to give the unsymmetrical urea, but the amine hydrochloride had to be treated twice with the reagent. Di-n-octadecylamine reacted with phenyl isocyanate and α-naphthyl isocyanate to give the corresponding urea derivatives; yet di-n-octadecylamine did not react with phenyl isothiocyanate. It is probable that this reagent is much less reactive than the phenyl isocyanate.

The desulfuration of N,N'-di-n-octadecylthiourea proceeded with surprising ease for a compound of this molecular weight. It is an indication of the lability of sulfur in this class of compounds.

The acylation of amines is one of their most important reactions. It is not surprising, therefore, that various procedures have been worked out in which different reagents were used to special advantage. Some of the more important methods of acylation are:

(a) $RNH_2 + R'CO_2H \longrightarrow R'CONHR + H_2O$

(b) $2\ RNH_2 + R'COCl \longrightarrow R'CONHR + RHN_2 \cdot HCl$

(c) $RNH_2 + (R'CO)_2O \longrightarrow R'CONHR + RCO_2H$

(d) $RNH_2 + R'CO_2R'' \longrightarrow R'CONHR + R''OH$

(e) $RNH_2 \cdot HCl + R'COCl \longrightarrow R'CONHR + 2\ HCl$

[15] Hoyt, Doctoral Thesis, "The Phenomenon of Homology With Long-Chained Aliphatic Compounds," Library, Iowa State College (1940).
[16] Flaschenträger and Lachmann, Z. physiol. Chem., 192, 268 (1930).
[17] Jeffreys, Ber., 30, 898 (1897); Am. Chem. J. 22, 31 (1899).
[18] French and Wirtel, J. Am. Chem. Soc., 48, 1736 (1926).

TABLE 1. *Urea and thiourea derivatives of n-dodecyl-n-octadecyl- and di-n-octadecylamines*

Compound	Formula	M.p.°C.	Nitrogen percentage		
			Yield[a]	Calcd.	Found
N,N'-di-n-dodecylthiourea	$C_{25}H_{52}N_2S$	74.5- 75.0	72	6.80	6.37
N-n-Dodecyl-N'-phenylthiourea	$C_{19}H_{32}N_2S$	69.5- 69.8	72	8.75	8.17
N-n-Dodecyl-N'-α-naphthylurea[b]	$C_{23}H_{34}ON_2$	127.5-128.0	64	7.91	7.42
N-n-Dodecylammonium N-n-Dodecylcarbamate	$C_{27}H_{56}O_2N_2$	85.5- 86.5	78	6.76	6.57
N,N'-Di-n-octadecylthiourea ..	$C_{37}H_{76}N_2S$	95.0- 96.0	80	4.83	5.06
N,N'-Di-n-octadecylurea	$C_{37}H_{76}ON_2$	112.0-112.5	87	4.96	4.79
N-n-Octadecyl-N'-α-naphthylurea[b]	$C_{29}H_{46}ON_2$	122.5-123.0	63	6.38	6.27
N,N-Di-n-octadecylurea	$C_{37}H_{76}ON_2$	65.0- 65.5	61	4.96	5.02
N,N-Di-n-octadecyl-N'-phenylurea	$C_{43}H_{80}ON_2$	56.0- 56.5	84	4.37	4.17
N,N-Di-n-octadecyl-N'-α-naphthylurea[b]	$C_{47}H_{82}ON_2$	54.0- 55.0	89	4.06	3.78

[a] The majority of the compounds crystallized well from either absolute ethanol or 95 percent ethanol.

[b] The crystals were stored in brown bottles since they turned pink on exposure to air and light.

It will be noticed at once that all the procedures are related in that an amine or its derivative is treated with an acid or its derivative to give the desired amide.

Reaction (a) is most direct, for it involves the reaction of an amine and a carboxylic acid to give the amide and water. This reaction may be called "the direct condensation of amines and carboxylic acids." Previous investigators only treated low-molecular-weight aliphatic amines in this fashion. The reaction was either carried out as a two-phase process, that is, the amine was neutralized with the acid, and the salt then decomposed by heat to the amide[19], or as a one-phase reaction in which the reagents were heated in sealed tubes[20].

It was found that the direct condensation could be applied to high-molecular-weight aliphatic primary amines and carboxylic acids with surprising ease; a necessary procedure was to heat the reagents in an open vessel at a temperature (usually 250°) where the water was evolved readily in the form of steam. When a stream of nitrogen was passed over

[19] Wurtz, *Ann.*, 76, 324 (1850); *Hofmann, Ber.*, 15, 977 (1882).

[20] Franchimont and Klobbie, *Rec. trav. chim.*, 6, 247 (1887); Tafel and Stern, *Ber.*, 33, 2232 (1900); Ruhoff and Reid, *J. Am. Chem. Soc.*, 59, 401 (1937).

TABLE 2. *Amides prepared by direct condensation of n-dodecyl- and n-octadecylamines and carboxylic acids*[a]

Compound	Formula	M.p. °C.	Reaction time (min.)	yield[b]	Nitrogen percentage	
					Calcd.	Found
N-n-Octadecylbenzamide	$C_{25}H_{43}ON$	85.0-85.5	60	89	c	4.36
N-n-Dodecyl-m-toluamide	$C_{20}H_{33}ON$	47.0-47.5	30	57	4.62	3.43
N-n-Octadecyl-m-toluamide	$C_{26}H_{45}ON$	71.0-71.5	45	78	3.62	3.43
N-n-Dodecyl-o-toluamide	$C_{20}H_{33}ON$	55.0-55.5	30	72	4.62	4.34
N-n-Octadecyl-o-toluamide	$C_{26}H_{45}ON$	73.5-74.0	30	76	3.62	3.47
N-n-Dodecylanisamide	$C_{20}H_{33}O_2N$	87.5-88.0	30	69	4.38	4.24
N-n-Octadecylanisamide	$C_{26}H_{45}O_2N$	100.0-100.5	30	63	3.47	3.03
N-n-Dodecyl-o-chlorobenzamide	$C_{19}H_{30}ONCl$	61.0-61.5	45	60	4.33	4.07
N-n-Octadecyl-o-chlorobenzamide	$C_{25}H_{42}ONCl$	78.0-78.5	45	44	3.44	3.34
N-n-Dodecyl-p-chlorobenzamide	$C_{19}H_{30}ONCl$	77.0-78.0	60	58	4.33	4.10
N-n-Octadecyl-p-chlorobenzamide	$C_{25}H_{42}ONCl$	94.0-94.5	25	58	3.43	3.36
N-n-Dodecylcinnamide	$C_{21}H_{33}ON$	73.0-73.5	30	55	4.44	4.24
N-n-Octadecylcinnamide[d]	$C_{27}H_{45}ON$	88.5-89.0	120	77	3.51	3.34
N-n-Dodecyllauramide[d]	$C_{24}H_{49}ON$	77.0-77.5	40	77	3.79	3.48
N-n-Octadecyllauramide	$C_{30}H_{61}ON$	84.5-85.0	60	92	3.11	2.95
N-n-Dodecylmyristamide	$C_{26}H_{53}ON$	83.0-83.5	20	79	3.54	3.18
N-n-Octadecylmyristamide	$C_{32}H_{65}ON$	87.5-87.8	60	80	2.92	2.68
N-n-Dodecylpalmitamide	$C_{28}H_{57}ON$	82.0-82.5	15	85	3.31	2.98
N-n-Octadecylpalmitamide	$C_{34}H_{69}ON$	90.0-90.5	30	66	2.76	2.58
N-n-Dodecylstearamide	$C_{30}H_{61}ON$	84.0-84.5	30	77	e	
N-n-Octadecylstearamide	$C_{36}H_{73}ON$	94.5-95.0	30	71	e	
N-n-Dodecyloleamide	$C_{30}H_{59}ON$	49.0-51.0	20	50	f	3.01
N-n-Octadecyloleamide	$C_{36}H_{71}ON$	70.0-70.5	15	59	2.62	2.48
N-n-Dodecylelaidamide	$C_{30}H_{59}ON$	73.5-74.0	25	83	3.12	2.68
N-n-Octadecylelaidamide	$C_{36}H_{71}ON$	83.5-84.0	20	78	2.62	2.48

[a] the reactions were conducted at 250°.

[b] ethanol, dilute ethanol, acetone and dilute acetone were the most frequent crystallizing solvents.

[c] mixed m.p. with an authentic specimen prepared from n-octadecylamine and benzoyl chloride (table 4).

[d] the reaction temperature was 200°.

[e] mixed m.p. with an authentic specimen prepared from n-dodecylamine hydrochloride and stearoyl chloride (table 4).

[f] mixed m.p. with an authentic specimen prepared by B. A. Hunter from n-octadecylammonium stearate.

the heated reagents, the discoloring was minimized, and the product was obtained more readily in a colorless crystalline state.

In this manner amides were prepared of n-dodecyl- and n-octadecylamines with various aromatic acids. Thus, amides of benzoic, o-toluic, m-toluic and anisic acids were obtained in average yields of 72 per cent. Moreover, it was possible to extend the condensation to chloro-substituted benzoic acids. While the p-chlorobenzamides were prepared with no difficulty, the o-chlorobenzamides were more difficult to purify (table 2).

The direct condensation of amines and carboxylic acids worked extremely well with high-molecular-weight aliphatic acids. This result was an important synthetic improvement, since the preparation of the troublesome acid chlorides then became unnecessary. In this manner, the n-dodecyl and n-octadecyl amides of lauric, myristic, palmitic and stearic acids were obtained in average yields of 78 per cent (Table 2).

The direct condensation of high-molecular-weight aliphatic amines could be extended to unsaturated aromatic and aliphatic acids without isomerization of the latter. The n-dodecyl and n-octadecyl amides of trans-cinnamic, cis-oleic and trans-elaidic acids were prepared in average yields of 67 per cent (table 2).

The direct condensation of amines and carboxylic acids was successfully extended to aliphatic diamines. 1,10-Decanediamine reacted with an equivalent of lauric acid to give the dilauramide.

An investigation of the mechanism of the direct condensation of amines and carboxylic acids showed that there was initial salt formation but under the general procedure adopted the salt was formed and immediately decomposed to the amide.

The direct condensation of amines and sulfonic acids was found to stop at the salt stage. The sulfonium salts were stable to heat, but like the ammonium salts had broad melting ranges (table 3).

TABLE 3. p-Toluenesulfonic acid salts

Compound	Formula	M.p.°C.	Nitrogen percentage		
			Yield	Calcd.	Found
n-Dodecylammonium p-toluenesulfonate	$C_{19}H_{35}O_3NS$	100–137	87	3.92	3.77
n-Octadecylammonium p-toluenesulfonate	$C_{25}H_{47}O_3NS$	93–138	98	3.17	2.98

The original Hinsberg technique[21] was found to apply to high-molecular-weight aliphatic amines. The acylation of high-molecular-weight alipathic amines with acetic, benzoic and phthalic anhydrides proceeded readily. The phthalimides were hydrolyzed with sodium hydroxide to the corresponding phthalmic acids (table 4).

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n-Octadecylamine reacted immediately with diethyl oxalate, while the reaction with malonic ester required heating (table 4). The reaction with diethyl ethylmalonate gave no pure product. This was in line with the results of Franchimont and Klobbie[22] on the effect of alkyl groups in the malonic ester molecule.

TABLE 4. *Amides prepared from acid chlorides[a] acid anhydrides[b] and esters[c]*

Compound	Formula	M.p.°C.	Nitrogen percentage		
			Yield	Calcd.	Found
N-n-Octadecylbenzamide[a]	$C_{25}H_{43}ON$	85.5- 86.0	43	3.75	3.98
N-n-Dodecylstearamide[a]	$C_{30}H_{61}ON$	84.5- 85.0	92	3.14	2.88
N-n-Dodecylbenzene-sulfonamide[a]	$C_{18}H_{31}O_2NS$	57.5- 58.0	67	4.31	3.72
N-n-Octadecylbenzene-sulfonamide[a]	$C_{24}H_{43}O_2NS$	77.0- 77.5	68	3.42	2.95
N-n-Dodecylacetamide[b]	$C_{14}H_{29}ON$	53.5- 54.0[d]	45	6.17	5.84
N,N-Di-n-octadecyl-benzamide[a] [b]	$C_{43}H_{85}ON$	55.0- 56.0	64 / 86	2.37	2.29
N-n-Dodecylphthalimide[b]	$C_{20}H_{29}O_2N$	64.0- 64.5	68	4.47	3.80
N-n-Dodecylphthalamic acid[c] .	$C_{20}H_{31}O_3N$	87.0- 88.5[d]	89	e	
N-n-Octadecylphthalimide[b] ...	$C_{26}H_{41}O_2N$	79.0- 79.5	66	3.51	3.14
N-n-Octadecylphthaamic acid[c].	$C_{26}H_{43}O_3N$	90.5- 92.5[d]	89	3.36	3.26
N,N'-Di-n-octadecyloxamide[c] .	$C_{38}H_{76}O_2N_2$	119.0-119.5	66	4.73	4.52
N,N'-Di-n-octadecyl-malonamide[c]	$C_{39}H_{78}O_2N_2$	126.0-126.2	50	4.62	4.53

[a] previously reported as a liquid b.p. 212-213°/13 mm.; English patent, 458, 454 (1937) [*Chem. Zentr., I*, 2867 (1937)].

[e] previously reported to melt at 88°; U. S. patent, 2,101,323 (1937) [*ibid., I*, 2061 (1938)].

The value of pairs of derivatives of n-dodecyl- and n-octadecylamines was studied (table 5). It was found that in all the cases listed, with the exception of the α-naphthylurea derivatives, there was either a marked average lowering of the mixed melting point and/or a marked increase in the melting point range.

If the derivatives were arranged in order of decreasing value they would be as follows: (1) N-acetamides, (2) N-o-toluamides, (3) N-benzenesulfonamides, (4) N-p-toluenesulfonamides, (5) N-anisamides, (6) N-cinnamides, (7) N,N'-phenylthioureas, (8) N-phthalimides, (9) N-myristamides, (10) N-p-chlorobenzamides, (11) N-elaidamides, (12) N-m-toluamides, (13) N-palmitamides, (14) N-stearamides, (15) N-lauramides and (16) N,N'-α naphthylureas. Although the largest depression

[22] Franchimont and Klobbie, *Rec. trav. chim., 4*, 195 (1885).

TABLE 5. *Mixed melting points of n-dodecyl and n-octadecyl derivatives*

Type compound	n-Dodecyl m.p. °C.	n-Octadecyl m.p. °C.	Mixed m.p. °C.	Average lowering °C.
N,N'-α-naphthylurea ...	127.5-128.0	122.5-123.0	121.5-123.0	3.5
N,N'-Phenylthiourea	69.5- 69.8	86.0- 87.0*	69.0- 80.0	8.5
N-Benzenesulfonamide ..	58.0- 58.5	77.0- 77.5	55.0- 59.0	12.5
N-p-Toluenesulfonamide	73.0- 73.5	88.0- 90.5*	69.5- 74.0	11.5
N-m-Toluamide	47.0- 47.5	71.0- 71.5	55.0- 65.0	4.0
N-o-Toluamide	55.0- 55.5	76.5- 77.0	52.5- 61.0	13.5
N-Anisamide	87.5- 88.0	100.0-100.5	82.5- 85.0	11.5
N-p-Chlorobenzamide ...	78.5- 79.0	94.0- 94.5	76.0- 84.5	6.0
N-o-Chlorobenzamide ..	62.0- 62.5	78.0- 78.5	53.5- 58.0	17.0
N-Cinnamide	73.0- 73.5	88.5- 89.0	70.5- 78.0	10.5
N-Phthalimide	64.0- 64.5	79.0- 79.5	64.0- 75.0	7.5
N-Acetamide	53.5- 54.0	76.0- 77.5*	50.5- 52.0	14.5
N-Lauramide	77.0- 77.5	84.5- 85.0	73.0- 74.0	7.5
N-Myristamide	83.0- 83.5	87.5- 87.8	78.5- 82.0	7.0
N-Palmitamide	82.0- 82.5	90.0- 90.5	81.0- 85.5	5.0
N-Stearamide	84.5- 85.0	94.5- 95.0	83.5- 87.0	5.0
N-Elaidamide	83.5- 74.0	83.5- 84.0	72.5- 80.0	6.0

* Specimens kindly supplied by F. W. Hoyt.

occurred with the o-chlorobenzamides they did not crystallize with ease. This offset their advantage as derivatives.

Several of the compounds prepared were found to be either soluble in water or have soap forming power. n-Dodecylamine hydrochloride and 1,10-decanediamine dihydrochloride were soluble in water. n-Dodecylammonium p-toluenesulfonate was moderately soluble in water with the formation of a detergent solution. The n-octadecyl analog formed a soapy suspension when shaken with water. The sodium salts of N-n-dodecyl- and N-n-octodecylphthalamic acids also had emulsifying properties.

The compounds described in this paper were analyzed, with few exceptions, by the Kjeldahl method. The sample (0.5 g.) was digested with 25 ml. of a selenium oxychloride solution made up by dissolving 8.0 ml. of selenium oxychloride in 992 ml. of concentrated sulfuric acid. More dilute solutions of selenium oxychloride gave low results.

<div align="center">EXPERIMENTAL</div>
<div align="center">PREPARATION OF NITRILES</div>

The following procedure was used in the preparation of stearonitrile: In a 1 l. Claisen flask was placed 284 g. (1.0 mole) of purified stearic acid. The long neck carried a two-hole stopper which contained a glass inlet

tube for the ammonia. This reached to the bottom of the flask. Through the other hole was inserted a thermometer also reaching to the bottom. The other neck of the flask was closed by a cork stopper while the distilling tube was left open for the escape of the ammonia. The distilling tube dipped into an empty beaker in order to catch the water and any material ejected over. The heating was done in a hood by means of a Méker burner. The flask was placed in a six-inch graphite bath, the large supporting ring of which was 8.5 in. from the ring stand. When the temperature was approximately 150° the inlet tube was connected with the ammonia tank through a bubbler of mineral oil to observe the rate of flow of ammonia. To maintain an excess of ammonia a steady stream of bubbles was maintained. The presence of excess ammonia was detected at the outlet of the distillation tube by means of a cotton dauber moistened with concentrated hydrochloric acid. The heating was conducted at 330° for 9 hours. The molten contents of the flask were transferred to a 1 l. Claisen flask with a 20 cm. fractionating column, and distilled under reduced pressure. It was found advantageous to place an asbestos guard around the distillation apparatus in order to exclude drafts. The nitrile boiled over a two degree range 185-187°/4 mm., with no forerun. The colorless distillate melted at 39-40°, and weighed 203-208 g. (77-78 per cent).

During the heating the water which was evolved partly refluxed back onto the hot acid causing a mild and occasional seething. It was necessary occasionally to wipe the distilling tube with a flame to melt the small amount of material which collected there and which may prevent the smooth removal of the water. This was the only attention the reaction required once the temperature had been reached and the ammonia rate fixed.

The product may be purified further by refluxing a short while with 1 l. of 2 per cent potassium hydroxide in 50 per cent ethanol, followed by two reflux-washings with plain 50 per cent ethanol. Finally, it was crystallized from 2 l. of 95 per cent ethanol at 0° to melt at 41.0-42.0°.

Lauronitrile (b.p. 130-136°/3 mm.) was prepared similarly in 73 per cent yield by heating at 270-275° for 12 hours. In the preparation of sebaconitrile (b.p. 168-170°/3 mm., $n_D^{25} = 1.4462$) an ordinary distilling flask was used. Heating was conducted at 220° for 6 hours, and the yield was 55 per cent.

PREPARATION OF 1,10-DECANEDIAMINE

To 16.4 g. (0.1 mole) of sebaconitrile in a Parr hydrogenation bomb was added 25 ml. of a Raney nickel suspension in petroleum ether (b.p. 60-68°). Ammonia gas was introduced up to a pressure of 160 lbs./sq. in. and then 500 lbs./sq. in. of hydrogen. The bomb was rocked, and the temperature raised to 140°. This temperature was maintained for 30 minutes. The greatest decrease in pressure occurred as the bomb reached 135°. By the time the temperature reached 140° there was no further decrease in pressure. The theoretical absorption of hydrogen took place. After cooling to room temperature, the bomb contents were taken up in

300 ml. of hot petroleum ether (b.p. 60-68°) and filtered hot from the catalyst. The solvent was removed, and the residue vacuum distilled. The apparatus was protected from carbon dioxide by attaching soda-lime towers at all outlets. The liquid boiled sharply at 122.0-123.0°/ 4 mm. The yield was 10.6 g. (62 per cent). The colorless distillate solidified in the receiver. The product, when quickly manipulated into a capillary tube, melted at 61.0-61.5°. The compound readily absorbed carbon dioxide from the air causing a rise in melting point. A sample of the amine after standing in an open container melted at 133-137°.

1,10-Decanediamine has been reported[23] to melt at 61.5°.

MANIPULATION OF HIGH-MOLECULAR-WEIGHT PRIMARY AMINES

It is imperative to avoid both carbon dioxide and moisture in the preparation. This has been done in several ways. One way is to convert the crude amine to the hydrochloride, and whenever free amine is required the calculated amount of calcium oxide is admixed with the hydrochloride, and the amine distilled in an apparatus containing soda-lime towers at all inlets. It is unwise to distil more than 250 ml. or an equivalent weight of amine at one time since the vapors attack the rubber stoppers and give a yellow odorous distillate. The rubber stoppers must be pretreated with hot sodium hydroxide to remove any free sulfur, and just before use immersed in chloroform for a few minutes to dissolve out superficial impurities.

The free amines may be stored in closely stoppered containers which are opened only for the short time required to weigh out a portion. Speed in manipulation is essential. For this purpose the amines should be manipulated in a liquid condition. This is best obtained by warming the container in the water bath until the contents are molten. Two beakers are balanced and then placed on the hot plate. The container is removed from the water bath and the warm beakers replaced on the balance. Slightly more than the required amount of amine is poured into the beaker and enough withdrawn with a warmed medicine dropper to give the desired weight. The container is immediately stoppered, and the weighed molten amine poured into the reaction flask.

PREPARATION OF AMINE HYDROCHLORIDES

The following procedure illustrates the technique for the preparation of amine hydrochlorides: To a solution of 74.0 g. (0.4 mole) of n-dodecylamine in 500 ml. of ethanol was added 68.0 g. (0.8 mole) of concentrated hydrochloric acid. The solution darkened and at 0° gave pinkish crystals which were washed with cold dilute ethanol. The colorless crystals melted at 181°. The yield was 62.0 g. (70 per cent).

n-Dodecylamine hydrochloride has been reported to melt at 100° with decomposition[24].

Anal. Calcd. for $C_{12}H_{28}NCl$: N, 6.32. Found: N, 5.98.

[23] Phookan and Krafft, *Ber.*, 25, 2252 (1892).
[24] Lutz, *Ber.*, 19, 1436 (1886).

PREPARATION OF PHENYLTHIOUREAS

The preparation of N-n-dodecyl-N′-phenylthiourea will illustrate the procedure: To 14.8 g. (0.11 mole) of phenyl isothiocyanate in a 250 ml. Erlenmeyer flask was added 18.5 g. (0.1 mole) of n-dodecylamine. The reaction was exothermic, and a clear yellow liquid was formed. After a few minutes, the flask was immersed in an ice bath, and the flask turned so that the material solidified in a thin layer. This material was pulverized and washed with 100 ml. of 50 per cent ethanol. A crystallization from ethanol at 0° gave fine colorless crystals melting at 69.5-69.8°. The yield was 23.0 g. (72 per cent).

PREPARATION OF PHENYLUREAS

The preparation of N,N-di-n-octadecyl-N′-phenylurea will illustrate the procedure: A 200 ml. round bottom flask was warmed in a Bunsen flame and closed with a stopper carrying a calcium chloride tube. In the cool flask was placed a solution of 5.2 g. (0.01 mole) of di-n-octadecylamine in 50 ml. of warm dry benzene, and 1.3 g. (0.11 mole) of phenyl isocyanate was washed in with small portions of the solvent. The solution was refluxed for an hour, protecting against moisture by means of a calcium chloride tube. After removal of the benzene, a colorless residue was obtained which melted at 52.0-54.0°. Crystallization from absolute ethanol gave colorless crystals which melted at 56.0-56.5°. The yield was 5.4 g. (84 per cent).

PREPARATION OF α-NAPHTHYLUREAS

The preparation of N-n-dodecyl-N′α-naphthylurea will illustrate the general procedure: A 125 ml. Erlenmeyer flask was dried by heating in a Bunsen flame, then closed with a stopper carrying a calcium chloride tube. After cooling to room temperature, 9.2 g. (0.05 mole) of molten n-dodecylamine was added, followed by the addition of 9.2 g. (0.055 mole) of α-naphthyl isocyanate. The reaction was exothermic. After cooling to room temperature and pulverizing, the tan solid was crystallized from 300 ml. of petroleum ether (b.p. 77-115°). Repeated crystallization from ethanol gave colorless plates melting at 127.5-128.0°. The yield was 10.4 g. (64 per cent).

PREPARATION OF N,N′-DI-N-OCTADECYLUREA

In a 500 ml. three-necked flask equipped with a dropping funnel, reflux condenser and mercury-sealed stirrer was placed 4.5 g. (0.0077 mole) of N,N′-di-n-octadecylthiourea dissolved in 150 ml. of hot absolute ethanol. The solution was stirred, and 2.87 g. (0.0169 mole) of silver nitrate solution was added. The latter solution was prepared by dissolving the silver nitrate in 5 ml. of water and adding 5 ml. of absolute ethanol just before use. The precipitation of silver sulfide was immediate. Also, some of the urea separated out. A solution of 0.95 g. (0.0169 mole) of potassium hydroxide dissolved in 20 ml. of 95 per cent ethanol was added, and the mixture refluxed with stirring for 30 minutes. It was then trans-

ferred to a 1 l. Erlenmeyer flask, and enough absolute ethanol added to dissolve the product. It was filtered hot from the silver sulfide. The filtrate deposited colorless crystals which melted at 112.0-112.5°. The yield was 3.5 g. (87 per cent).

DIRECT CONDENSATION OF AMINES AND CARBOXYLIC ACIDS

The preparation of N,N'-decamethylenedilauramide will illustrate the general technique: In a 125 ml. Erlenmeyer flask was placed a mixture of 5.2 g. (0.03 mole) of 1,10-decanediamine and 12.0 g. (0.06 mole) of lauric acid. A glass inlet tube was placed above the surface of the molten mixture, and nitrogen passed in through a mineral oil bubble counter. After 5 minutes, the flask was inserted in a metal bath held at 195°, maintaining a steady flow of nitrogen bubbles during the heating. In a short while there was some evidence of reaction. Volatilization and discoloration were slight. The heating was continued for 1 hour. After cooling to room temperature, the tan solid was crystallized from absolute ethanol at 0° to give the constant melting point of 137.0-137.5°. The yield was 13.7 g. (85 per cent).

Anal. Calcd. for $C_{34}H_{68}O_2N_2$: N, 5.22. Found: N, 4.96.

MECHANISM OF THE DIRECT CONDENSATION OF AMINES AND CARBOXYLIC ACIDS

To 6.0 g. (0.03 mole) of lauric acid in a 125 ml. Erlenmeyer flask was added 8.1 g. (0.03 mole) of n-octadecylamine, and the mixture placed in an oil bath at 65° for 15 minutes. The contents of the flask remained crystalline and colorless. In the next 20 minutes, the temperature was raised to 100°. The molten contents were agitated and cooled to room temperature. The colorless solid melted at 64.0-65.5° and weighed 14.1 g. The yeild was quantitative. A crystallization from petroleum ether (b.p. 60-68°) gave colorless plates melting at 65.5-66.5°. A mixed melting point with an authentic specimen of n-octadecylammonium laurate melting at 66.6-67.0°[25] gave no depression.

PREPARATION OF PHTHALMIDES

The preparation of N-n-dodecylphthalimide will illustrate the procedure: To 24.4 g. (0.165 mole) of phthalic anhydride in a 500 ml. round bottom flask was added 27.8 g. (0.15 mole) of n-dodecylamine. A thermometer was inserted in the reaction mixture and the contents heated at 200° for 5 minutes. By this time the evolution of steam was complete. The molten mass was poured into a large evaporating dish and turned so that the liquid solidified in a thin layer. The dull, white solid melted at 58-60.5°. A portion of this was crystallized from ethanol and Norit at 0° to give colorless plates melting sharply at 64.0-64.5°.

PREPARATION OF PHTHALAMIC ACIDS

The preparation of N-n-dodecylphthalamic acid will illustrate the procedure: To 30.0 g. (0.095 mole) of crude N-n-dodecylphthalimide in a

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*Kindly supplied by B. A. Hunter.

large evaporating dish was added 500 ml. of 10 per cent sodium hydroxide, and the mixture heated on the water bath with frequent stirring for 1 hour. Upon addition of 2 l. of distilled water a clear solution formed. Acidification with dilute hydrochloric acid gave a copious precipitate which was filtered and washed with distilled water. The colorless fine crystals melted at 87.0-88.5° with decomposition to the imide.

PREPARATION OF N,N'-DI-N-OCTADECYLMALONAMIDE

To 8.8 g. (0.55 mole) of diethyl malonate in a 125 ml. Erlenmeyer flask was added 26.9 g. (0.1 mole) of n-octadecylamine. The flask was closed with a stopper carrying a soda-lime tube and inserted in an oil bath temperature of 110°. Heating was continued for 1 hour. The molten contents were transferred to a large evaporating dish and turned so that the liquid solidified in a thin layer. The product was crystallized from petroleum ether (b.p. 77-115°) and gave a poor melting point of 122.5-123.5°. To the solid in a 1 l. Erlenmeyer flask was added 400 ml. of 85 per cent ethanol containing 10 ml. of concentrated hydrochloric acid. The mixture after refluxing a short while was filtered through a hot Buchner funnel and washed with hot 80 per cent ethanol. The product now melted at 125.5-126.5°. The yield was 15.1 g. (50 per cent). Crystallization from petroleum ether (b.p. 77-115°) raised the melting point to 126.0-126.2°.

REACTION OF AMINE HYDROCHLORIDES AND ACID CHLORIDES

The preparation of N-n-dodecylstearamide will illustrate the general procedure: In a 1 l. round bottom flask was placed 24.4 g. (0.11 mole) of n-dodecylamine hydrochloride. A solution of 30.2 g. (0.1 mole) of freshly prepared stearoyl chloride in 350 ml. of dry toluene was added, and the mixture refluxed for 24 hours. The condenser was capped with a calcium chloride tube. After cooling to room temperature, the solution crystallized and was further cooled to 0°. The colorless crystals melted at 83.0°. A crystallization from ethanol at 0° gave a colorless crystalline solid with wax-like characteristics melting at 84.5-85.0°. The yield was 41.6 g. (92 per cent).

Anal. Calcd. for $C_{30}H_{61}ON$: N, 3.14. Found: N, 2.88.

SUMMARY

The ammonolysis of high-molecular-weight aliphatic acids to nitriles has been improved and extended to aliphatic dibasic acids.

1,10-Decanediamine has been prepared by catalytic hydrogenation of sebaconitrile.

A series of urea and thiourea derivatives of n-dodecyl-, n-octadecyl- and di-n-octadecylamines has been prepared.

A general technique for the direct condensation of high-molecular-weight aliphatic primary amines and carboxylic acids has been developed, and extended to high-molecular-weight aliphatic acids, substituted and unsubstituted aromatic acids and aromatic and aliphatic olefinic acids. The mechanism of this condensation has been described.

The value of a number of compounds of the n-dodecyl and n-octadecyl series as derivatives has been determined.

The condensation of n-octadecylamine and some aliphatic dibasic esters has been accomplished.

The preparation of compounds of the n-dodecyl and n-octadecyl series either soluble in water or possessing detergent action has been successfully carried out.

Some suggestions were made for the analysis of compounds of high molecular weight.

BIOLOGICAL STUDIES OF ORNITHOCORIS TOLEDOI PINTO, THE BRAZILIAN CHICKEN BEDBUG

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The Brazilian chicken bedbug, *Ornithocoris toledoi* Pinto (Cimicidae, Hemiptera), was described by Cesar Pinto in 1927 from specimens collected in poultry houses in the vicinity of Limeira, São Paulo. In his description of the species, he cites the following characteristics (3).

Length: ♂ — 4.050 mm.; ♀ — 4.400 mm. Width of abdomen: ♂ — 2.050 mm.; ♀ — 2.250 mm. General color brown. Head pyramid-shaped, 500μ long by 750μ wide, including the eyes. Second antennal segment longer than the third. Body covered with short hairs. Protonum trapezoidal, with two spines of unequal length inserted in the posterior angles. Elytra squamiform, with three or four spines near the apex. Eighth abdominal segment of the male almost symmetrical. Protibiae and mesotibiae shorter than the metatibiae, wider at the apex. Inner surface of the distal extremity of protibiae and mesotibiae provided with a distinctive organelle constituted of a tuft of slightly recurved fine hairs. (See *A*, Plate I.)

Morais (2) in 1939 gives some additional morphological characters based on material collected in the state of Minas Gerais: Labrum with three symmetrical pairs of small spines; scutellum triangular, anterior margin almost straight, lateral margins slightly curved; tibial tuft present on fore and middle legs of the male; mesotibiae of the female without tuft; first abdominal segment indistinct; dorsal surface of second segment uniformly pilose; pilosity of segments 3 to 8 limited to posterior portions; apex of abdomen slightly asymmetrical.

DISTRIBUTION

Ornithocoris toledoi has been reported from two widely separated regions of Brazil. In addition to the original record from Limeira in the state of São Paulo, by Cesar Pinto in 1927, Carvalho verified the presence of this bedbug in poultry houses in the vicinity of Ponte Nova, Minas Gerais, in 1937 (1). At present the genus *Ornithocoris* includes only two species: *O. toledoi*, parasite of *Gallus domesticus*, and *O. furnarii* (Cordero et Vogelsang, 1928), found in the nests of *Furnarius rufus* in Uruguay. In the opinion of Cesar Pinto, these species are probably identical (3).

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BIOLOGICAL STUDIES

Very little of the biology of *Ornicothoris toledoi* has been reported since the description of the species. As this bedbug is a serious pest of chickens within its known geographic range, increased knowledge of its developmental cycle and habits will be of general interest to parasitologists, as well as of value in formulating practical measures for its control.

In 1938, a number of specimens were collected in poultry houses near Ponte Nova and brought to the State University of Agriculture and Veterinary Science of Minas Gerais, Vicosa, Brazil. From this original stock, a supply of several hundred individuals has been successfully maintained, under laboratory conditions, during 1939. The insects were reared in wooden or glass containers, screened to provide adequate ventilation. These cages were approximately 25 x 12 x 15 in., and large enough to allow free movements of host birds on which the bedbugs normally feed. Small pieces of wood, folded cardboard and bones were found to provide excellent conditions for deposition of eggs as well as ideal hiding places for nymphs and adults.

Individuals under observation for copulation, egg counts, incubation period, stadia and ecdyses were confined in Petri dishes lined with filter paper and covered with cheese cloth. Practically all investigations were conducted under natural environmental conditions of temperature and relative humidity. Temperature and humidity records taken in the laboratory and in poultry houses were almost identical throughout the period of the investigations. The data presented are from laboratory studies supplemented by observations made in infested poultry yards.

HABITS: Nymphs and adults of *Ornithocoris toledoi* were found in chicken houses, and in various situations in the immediate vicinity of isolated roosts when no adequate poultry houses were provided. Occurrences have been verified in the walls and other suitable hiding places in human dwellings where chickens were permitted to roost. Occupants of such houses report that they have suffered no inconvenience under these conditions, as this bedbug apparently does not feed on man.

Hiding places in chicken houses are of the usual variety. The authors have found specimens in cracks between boards of walls, floors, ceilings, doors and windows; in small openings and in the joints of roosts and nest boxes; under nest boxes, loose boards, sacks, cans, trash, etc. In brick buildings, a few individuals were found in accessible chinks between the bricks of the walls, but the greater number seem to prefer the wooden parts of these constructions. In houses having brick walls and cement floors, most bugs were localized in cracks in the boards of nest boxes and roosts.

Both nymphs and adults exhibit decided negative phototropism. This tendency to avoid both natural and artificial light, and the relatively small size of the insect, make discovery of the begbug slightly difficult, even in heavily infested poultry houses.

FEEDING: Under natural conditions, *Ornithocoris toledoi* was found to feed only at night. However, when nymphs and adults had access to

host birds under laboratory conditions, some individuals were found on the host at practically any hour of the day. The same condition was ob served in chicken houses when the interior was artificially darkened during the day.

Preparatory to feeding, the bug either passes directly from the nest to the body of the chicken or crawls up the feet and legs from the roost. In most cases there is a preference for the more vascular regions of the host's body. Feeding punctures are evidently painful, as host birds show marked reactions coincident with the insertion of the mouth parts. The parasites remain in the feeding position from 6 to 12 minutes. After taking a meal, nymphs either fall from the host or, together with the adults, crawl down the legs of the bird and search out suitable hiding places. At this time they are swollen with blood, reddish in color, and decidedly less active.

Feeding punctures sometimes provoke a slight hemorrhage, and always cause hyperaemia of the attacked area. This latter symptom persists some 12 hours after the attack, then gradually disappears.

Under experimental conditions, both adults and nymphs attacked and fed on turkeys, ducks and pigeons. Chickens, however, are the preferred host. When two birds were placed together in the breeding cages, the chicken was always attacked by the greater number of bedbugs, leaving the turkey, duck or pigeon practically unmolested. There was no apparent preference between turkeys and ducks, while pigeons were least attacked. No cases have been recorded in which nymphs or adults of *Ornithocoris toledoi* fed on man, either in infested chicken houses or, experimentally, in the laboratory, even under starvation conditions.

LIFE CYCLE: All developmental cycle data reported in the following pages are from individuals permitted to feed on *Gallus domesticus*.

Adults

Adult bugs visited the host at 8 day intervals, with small individual variations. They stayed on the body of the bird from 9 to 13 minutes and remained in the feeding position for 8 to 12 minutes. Digestion of the meal was completed in 3 to 4 days.

Ornithocoris toledoi is apparently less resistant to unfavorable environmental conditions than *Cimex lectularius* or *C. hemipterus*. High mortalities were observed in slight excesses of heat and cold, and in transporting the insects from one locality to another. This species also appears less able to survive long periods under starvation conditions. It was noted that males lived longer than females under normal conditions, and also in the absence of food.

Mating

No cases of copulation were recorded among adult bugs which were not permitted to feed after the fifth molt. Apparently a meal of blood is essential before mating can take place. The act of copulation usually oc-

curred within a 5 minute interval immediately following termination of the feeding period. When a number of recently fed adults of both sexes were confined in a small cage, more than one male attempted to mate with a single female at the same time. In copulation, the male mounts the female from behind, maintains his position by holding the female with his legs, and inserts the phallus in the triangular copulatory pouch located in the posterior margin of the fourth abdominal sternite ["organ of Ribaga and Berlese" of some authors— (3)]. Copulation lasts from 2 to 5 minutes. The male immediately leaves the female and searches out a suitable hiding place, while the female remains stationary for a short time.

As observed in these studies, females mated only once and were inaccessible to the males thereafter.

Egg Laying

Egg laying begins 5 to 8 days after copulation. Most eggs were deposited in cracks in or between boards, when such places were available. Eggs are laid in small groups of 2 to 4 in the form of a sticky mass which is securely glued to the surface of the wood. An average of four separate deposition periods (with variations from 2 to 6) was observed under laboratory conditions. Egg laying continued during 24 to 36 hours, with a normal range of 8 to 14 eggs during this time. Deposition periods were spaced at 6 to 10 day intervals with an average of 8 days between periods. The mean number of eggs per female was 46, with large individual variations ranging from 8 to 74.

The eggs of *Ornithocoris toledoi* are roughly oblong in shape, measuring approximately 700 μ in length by 370 μ in width at the widest diameter. (See *B*, Plate I.) When newly laid they are yellowish-white in color but soon darken as the development of the embryo progresses, and appear brown or light brown immediately before hatching. The shell is quite hard and resistant, and is provided with a distinct operculum.

Observations showed that a meal of blood is necessary between deposition periods if normal oviposition is to take place. This meal is usually taken immediately after completion of the preceding deposition period. To verify the relation between feeding and deposition, two groups of adult females that had initiated egg laying were not allowed to visit the host and were confined under starvation conditions. A few eggs were laid at irregular intervals, but no definite deposition periods occurred, in contrast to other groups which had free access to host birds and continued egg laying at well defined intervals.

Upon completion of the last definite deposition period, females lived from 3 to 7 months, visiting the host at increasingly longer intervals and remaining less time in the feeding position. A few eggs appeared at irregular intervals, but these were usually laid separately and were appreciably smaller than normal.

Incubation

The average incubation period as observed under laboratory condi-

tions (mean temperature 20.5° C., mean relative humidity 80 per cent) was 7½ days, with a normal range of 5 to 10 days. These conditions were practically identical with field conditions of temperature and relative humidity measured in the aviary during the same period.

The influence of temperature on length of incubation period is indicated by results of preliminary experiments. Various egg samples, placed in a constant temperature cabinet maintained at 25° C. and 80 per cent relative humidity, hatched in 110 to 120 hours, with no significant difference between means of incubation times at this temperature. Further investigtaions are now in progress to determine optimum and lethal temperatures for the eggs of *Ornithocoris toledoi*.

In hatching, (See C, Plate I) the young nymph forces open the operculum by exerting pressure with the cephalic region of the body. Recently hatched bugs usually remain near the egg mass, at times forming agglomerations of nymphs, shells and eggs. A few hours after hatching, the small nymphs search out suitable places of concealment, where they remain hidden until ready to take their first meal.

Nymphs

The young nymphs are yellowish-white in color, with legs and antennae disproportionately long in relation to the length and width of the body. In general appearance (Figure 1), they are similar to minute whitish mites. A short description of the first instar nymph follows: length, 0.853 ± 0.01 mm.[3]; width of abdomen, 0.443 ± 0.008 mm.; diameter of the head, 287-290µ; antennal index 4-2-3-1 (from longest to shortest segment); antennae pilose, with definite rows of small spines; clypeal suture indistinct; clypeus with a large spine in the anterio-lateral angles; eyes distinct and relatively large; thoracic tergites with a long spine in the posteriolateral angles; pronotum with two rows of small spines, one anterior, the other posterior; mesonotum and metanotum with a single transverse row of spines close to the posterior border; legs marked with definite rows of small spines; tibial tufts absent in both sexes; tarsi 2-segmented: tarsal claws relatively large; abdominal segments with a transverse row of spines; last abdominal segment with two pairs of long spines.

Under laboratory conditions, a number of first instar nymphs fed on the host within a few hours after hatching. However, under natural conditions, young nymphs did not visit the host until 20 to 28 hours after leaving the egg. Duration of the first nymphal feeding period is from 5 to 6 minutes. At the end of this period the ingested blood gives the nymph a reddish color; the abdomen is greatly swollen; and the young bugs frequently fall from the body of the host. Individuals which were allowed to feed immediately after hatching visited the host twice during the first, stadium. Others, kept one to three days without food, took a single meal before molting.

Three to 4 days are necessary for digestion of a meal. During this

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[3] Standard error.

period, fecal pellets are passed frequently, and the nymph gradually loses the reddish color. The average duration of the first stadium (at 20° C. and 80 per cent relative humidity) was 6 to 7 days. Variations ranging from 4 to 9 days were the general rule, but slightly longer periods were recorded for a few individuals.

In the majority of cases, a white spot appears at the posterior extremity of the abdomen some 24 to 48 hours preceding the first molt. The body gradually assumes a general whitish color; the exoskeleton ruptures in the region of the head capsule; and the second instar nymph slowly emerges. The process of leaving the exuvium usually necessitates approximately 24 hours in all ecdyses.
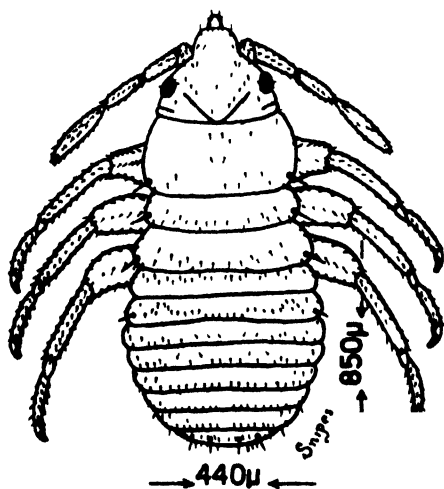
Fig. 1. Drawing of first instar nymph of *Ornithocoris oledoi*.
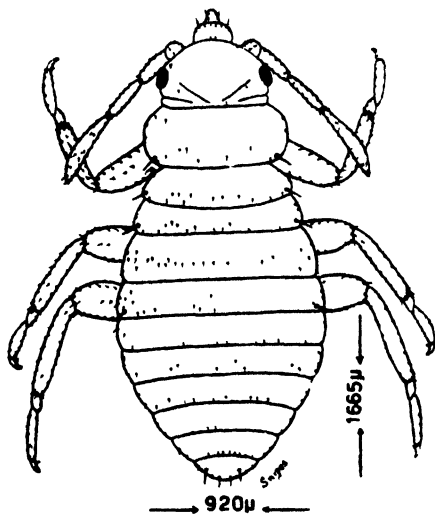
Fig. 2. Drawing of second instar female nymph.

Second instar nymphs (figure 2) measures 1.665 ± 0.009 mm. in length by 0.915 ± 0.085 mm. in width of abdomen. The diameter of the head varies from 330 to 369 μ. Second instar nymphs differ from the preceding instar in having the clypeal suture distinct, and in possessing tibial tufts on the prothoracic legs of the female and on the prothoracic and mesothoracic legs of the male. This second stage also visits the host one or two times, depending on the time of first feeding. Individuals that fed soon after ecdysis usually returned to the bird three or four days later for a second meal. Duration of the second stadium was 6 to 7 days under laboratory conditions, with variations ranging from 5 to 14 days.

Third instar nymphs are appreciably more active, but greatly resemble the preceding instar in general appearance. The body measures 1.906 ± 0.033 mm. in length and 1.051 ± 0.022 mm. in width of abdomen. The width of the head·capsule varies from 471 to 490 μ. Under laboratory conditions, all third instar nymphs fed at least twice, while a number of

individuals visited the host three times before molting. The average duration of the third stadium was 6 to 8 days, with a normal range from 5 to 14 days.

Fourth instar nymphs measure 2.369 ± 0.085 mm. in length by 1.474 ± 0.057 mm. in width of abdomen. The diameter of the head capsule varies from 512μ to 530μ. The fourth and fifth stadia averaged 6 to 8 days, with variations ranging from 4 to 13 days. Nymphs of these instars visited the host two or three times in each stadium. The last instar nymph measures 3.528 ± 0.071 mm. by 2.126 ± 0.018 mm., with head capsule measurements varying from 676μ to 685μ. In some cases the second tarsal suture was distinctly apparent in the fifth instar, while in others the tarsi did not appear 3-segmented until the fifth molt. Adult bugs (figure 3) are easily distinguished from late instar nymphs by the presence of the rudimentary whitish elytra.
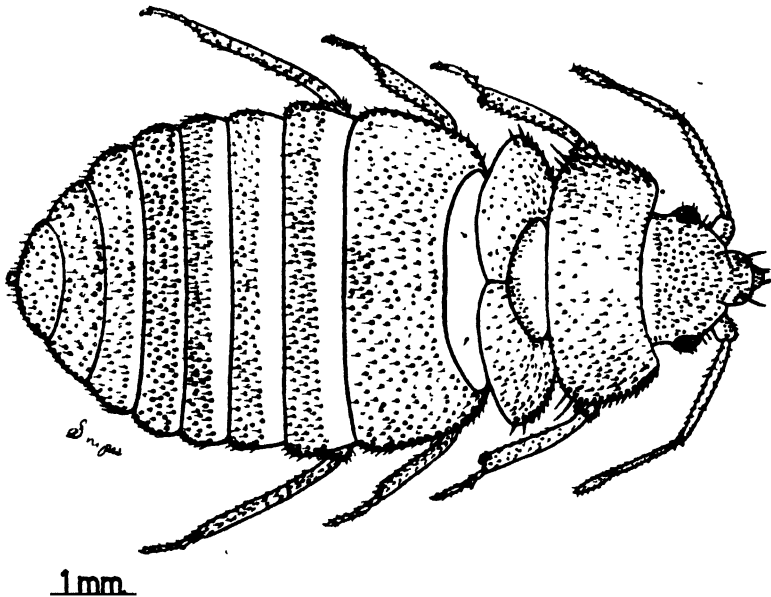
Fig. 3. Drawing of adult female of *Ornithocoris toledoi*.

The average difference in diameter of head capsule between nymphs of succeeding instars was found to be 0.81. This factor was used in determining instars and in verifying ecdysis counts.

Table 1 summarizes preliminary data recorded from studies on the life history of *Ornithocoris toledoi*.

DISCUSSION

Although the geographic distribution of *Ornithocoris toledoi*, as shown by existing records, includes only two widely separated localities,

it is thought that the insect is more widely distributed than these records indicate. It is a serious pest of chickens within its known range. The presence of this parasite in poultry houses is accompanied by large decreases in egg production and apparently by increased susceptibility to

Further studies on the biology and ecology of this insect are now in progress, together with experiments in the relative efficiency of various chemical control measures. The possible role of *Ornithocoris toledoi* as a disease vector is also being investigated.

TABLE 1. *Summary of developmental stages of Ornithocoris toledoi*

Stage, instar or period	Size			Duration of stage, instar or period	Feeding	
	Length	Width	Diameter of head		Number of meals	Interval
Egg	700μ	370μ	—	7.5 days (5-10)	—	—
1st Instar	0.853± 0.010 mm.	0.443± 0.008 mm.	287-290μ	6.5 days (4-9)	1-2	4-8 days
2nd Instar	1.665± 0.009 mm.	0.915± 0.085 mm.	330-369μ	6.5 days (5-14)	1-2	4-7 days
3rd Instar	1.906± 0.033 mm.	1.051± 0.028 mm.	471-490μ	7.0 days (5-14)	2-3	3-5 days
4th Instar	2.369± 0.085 mm.	1.474± 0.057 mm.	512-530μ	7.0 days (4-13)	2-3	3-5 days
5th Instar	3.528± 0.071 mm.	2.126± 0.018 mm.	676-685μ	7.0 days (4-13)	2-3	3-5 days
Hatching— Adult	—	—	—	34 days (22-63)	8-13	3-8 days
Adult	4.050 mm.— 4.400 mm.	2.050 mm.— 2.250 mm.	758-772μ	3-9 mos.	Variable	7-10 days
5th Molt to deposition	—	—	—	8.0 days (7-9)	1	—
Life cycle	—	—	—	48 days (36-85)	9-14	3-10 days

SUMMARY

This paper reports results of preliminary studies on the biology of the Brazilian chicken bedbug, *Ornithocoris toledoi* Pinto, a serious pest of chickens in Limeira, São Paulo, and in Ponte Nova, Minas Gerais, Brazil. Under experimental conditions this insect also fed on turkeys, ducks and pigeons, but did not attack man. Data reported include description of the egg, nymphs and adults; feeding habits; mating; deposition; incubation time; and duration of stadia.

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PLATE I

A, Photomicrograph of tibio-tarsal articulation, showing tibial tuft (400x) on prothoracic leg of adult male of *Ornithocoris toledoi*. *B.*, Drawing of 48-hour egg. C, Photomicrograph of 7-day eggs, showing escaping nymphs (72x).

PLATE I
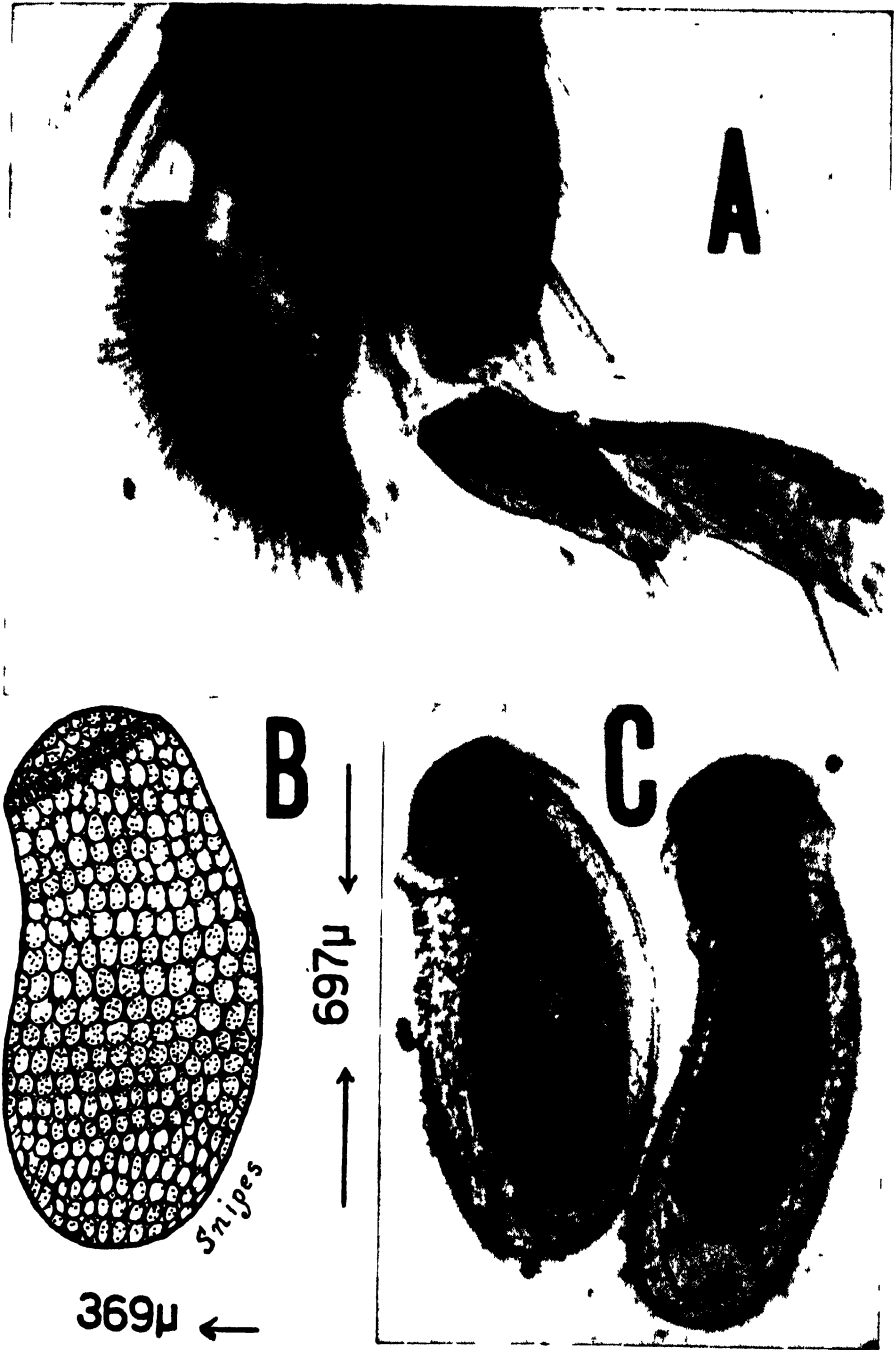
THE OXIDATION OF i-INOSITOL BY THE ACTION OF ACETOBACTER SUBOXYDANS[1]

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In a previous communication from these laboratories (1) preliminary studies were presented on the oxidation of i-inositol by means of *Acetobacter suboxydans,* and it was stated that "it was thought that if this compound (i-inositol) could be biologically oxidized to ketone compounds, which might exist in reversible oxidation-reduction systems, some light might be thrown upon its role as Bios I." The authors found that the culture could not be transferred beyond the third transfer in an inositol yeast extract medium. However, the addition of as little as 0.1 per cent of sorbitol to the medium permitted indefinite subculture and oxidation of the inositol. Results, to date, indicate the oxidation product to be principally a diketo-i-inositol. The complexity of the problem of positive identification of the ketoses is seen from the fact that there exists the possibility of 4 monoketo-i-inositols and of 9 diketo-i-inositols. Pasternak (7) described the preparation of a monoketo-inositol by the oxidation of i-inositol with nitric acid and gave the name inosose to the compound. Recently Kluyver and co-workers (5) (6) claim the production of inosose by the action of *Acetobacter suboxydans* upon i-inositol. None of the authors of this study attempted an identification of inosose among the three possible monoketo-i-inositols. The compound prepared in our laboratories is not inosose. It is possible that the differences in the results in our work and that of Kluyver lie in the use of different media, of a slightly different variety of the organism or a combination of both factors. Work is in progress to resolve the discrepancies just mentioned.

The present communication deals with studies on the development of optimum conditions for the oxidation of i-inositol, especially with the role of sorbitol and other compounds in the oxidation.

METHODS

The culture of *Acetobacter suboxydans* was obtained from the American Type Culture Collection listed as No. 621. The stock cultures are carried on 5 per cent sorbitol (or glycerol)-0.5 per cent yeast extract (Difco) agar slants. In several years experience with the culture no significant variations in growth or chemical action have been noted. In the work to be described the stock culture was transferred to a 10 per cent sorbitol-0.5 per cent yeast extract medium and kept active by transferring

each 3 days to a fresh medium. The medium will hereafter be referred to as the Basal Sorbitol Medium. The Basal Inositol Medium contained 3 per cent i-inositol, 0.05 per cent sorbitol and 0.5 per cent yeast extract; this medium permitted indefinite subculture of the organism. The Test Inositol Medium contained 3 per cent i-inositol and 0.5 per cent yeast extract.

Inoculations were made by adding 0.4 ml. of an active culture to each 10 ml. of medium in 125 ml. Erlenmeyer flasks. The medium was sterilized for 30 minutes at 20 lbs. steam pressure. The incubation temperature was 28°C. Fulmer, Dunning, Guymon and Underkofler (2) and Underkofler and Fulmer (9), in their studies on the production of sorbose and of dihydroxyacetone, noted that disturbing the cultures materially reduced yields. The same phenomenon was noted with the oxidation of inositol. Therefore, the technique of these authors was adopted and each analysis was made on a separate flask.

The course of the oxidation of the i-inositol was followed by use of the Shaffer-Hartmann (8) method. Since sorbose is formed in all cases involving the presence of sorbitol, the sorbose factor (1.25) was used to obtain comparative values and to correct for sorbose formed. That is, the ketose formed from the i-inositol is calculated as sorbose and the results listed in the tables simply as ketose. In each case in which sorbose might be present, the correction assumes a 100 per cent conversion of the sorbitol.

EXPERIMENTAL
PRELIMINARY

A three-day culture of *Acetobacter suboxydans,* grown on 50 ml. of the basal sorbitol medium, was filtered through a sterilized Berkefeld filter into a sterile suction flask. The filtrate was aseptically removed to a flask and used as inoculum *B*. The cells retained by the filter were washed several times with sterile physiological salt solution until completely freed from the medium. The washed cells were then suspended in 50 ml. of sterile salt solution and used as inoculum *A*. One ml. of a mixture of *A* and *B* was employed as inoculum *C*. Inoculum *D* consisted of another undisturbed culture. One ml. of each of the inocula was introduced into Medium I and Medium II, the composition of which, together with yields of ketose after 26 days incubation, are given in table 1. It is evident that

TABLE 1. *Comparison of different inocula*
(Grams "ketose" per 100 ml. of medium after 25 days)

Inoculum	Medium I	Medium II
A. Washed cells	2.75	0
B. Filtrate	0	0
C. Washed cells + filtrate	2.55	2.70
D. Culture	2.43	2.80

Medium I—5 g. i-inositol,,0.1 g. sorbitol, 0.5 g. yeast extract per 100 ml.
Medium II—5 g. i-inositol, 0.5 g. yeast extract per 100 ml.

the washed cells are unable to oxidize the inositol in the absence of the filtrate, the filtrate itself being inactive. The results show that residual sorbitol, or a product derived therefrom, was required for the organism to oxidize the inositol.

In order to further test the action of the medium-free cells upon the inositol, 15 ml. of a three-day culture of the organism, grown on the basal sorbitol medium, were centrifuged at 1500 RPM in a sterile 20 ml. centrifuge tube. The cells were then suspended in sterile salt solution and re-centrifuged until free from the medium and then suspended in sterile distilled water. One ml. of this suspension was used to inoculate a basal sorbitol medium and a test medium. The remaining 13 ml. were transferred to a flask in which 10 ml. of test medium had been allowed to evaporate to dryness at room temperature. Only the sorbitol medium supported growth; that is, even a massive inoculation of the medium-free cells was unable to oxidize the inositol. These results indicate that the organism is capable of oxidizing the inositol only when actively growing in the undisturbed culture.

THE EFFECT OF VARYING CONCENTRATIONS OF SORBITOL

In table 2 are presented data showing the effect of varying concentrations of sorbitol upon the yield of ketose. The inocula consisted of medium-free cells prepared by centrifugation and washing as described in the foregoing paragraph. While there is some action in very low concentrations, the data show a distinct drop in yield below 0.025 per cent sorbitol.

TABLE 2. *Effect of concentration of sorbitol upon yield of ketose (g. per 100 ml. of medium)*

Sorbitol, g. per 100 ml.	Days					
	1	2	3	4	5	6
0.100	..	0.52	1.42	....	1.43	1.40
0.075	....	0.47	1.37	1.42	1.45	1.45
0.050		0.46	1.10	1.46	1.45	1.45
0.025	...	0.26	0.74	1.02	1.31	1.34
0.010	0.03	0.07	0.20	....	0.35	0.38
0.001	0.08	....	0.15	....	0.17	0.18
0.0001	0.04	....	0.07	....	0.06	0.09

Since many inoculations of the above type would be necessary in subsequent work, experiments were undertaken to develop a medium containing sufficient sorbitol for continuous subculture but insufficient to permit the oxidation of the inositol in the test medium, when transferred to it. The above requirement was met by the use of 0.05 per cent of sorbitol. Results with inocula so prepared were identical with those obtained with the centrifuged and washed cells. This basal inositol medium

contains, per 100 ml., 3 g. i-inositol, 0.05 g. sorbitol and 0.5 g. yeast extract (Difco).

DEVELOPMENT OF THE OPTIMUM MEDIUM

In table 3 are given data showing the effect of varying surface-volume ratios upon the yield of ketose in the basal inositol medium. By surface-volume ratio is meant the square centimeters of surface per 100 ml. of medium. While the ratio, in the range studied, does not markedly influ-

TABLE 3. *Effect of surface-volume ratio upon yield of ketose on basal inositol medium*

	Surface-volume ratio					
Days	5.10	2.83	1.95	1.15	0.94	0.65
1	0.21	0.22	0 20	0.20	0.19	0.15
2	0.54	0.74	0.80	0.89	0.68	0.59
3	0.82	1.08	1.13	1.27	1.28	1.05
5	1.36	1.37	1.34	1 44	1.36	1.24
7	1 42	1.51	1.51	1 53	1 51	1.36

ence final yields, it is interesting to note that the rate of action is at a maximum at a ratio of 1.15. This result is surprising in view of the fact that in other oxidations by this organism studied in these laboratories, the rate of reaction increased with increasing surface-volume ratio.

The pH of several portions of the basal inositol medium was adjusted to several values by the use of either sodium hydroxide or of hydrochloric acid. The pH of the medium, as prepared, was 6.1. The pH of the media was determined again after sterilization. Since the pH changed somewhat during sterilization, the pH values listed in table 4 are those determined after sterilization. It is evident that pH values from 5.2 to 6.5 have very little influence upon the rates or final yields; pH values above

TABLE 4. *Effect of pH upon yield of ketose on basal inositol medium*

	pH (after sterilization)						
Days	4.3	5.2	5.3	6.1	6.5	7.0	7.6
1	....	0.12	0.08	0.19	0 23	0.03	0
2	0.07	0.93	0.79	0.86	0.90	0 45	0
3	0.10	....	. ..	. ..	1.18	....	0.08
4	....	1.30	1.31	1.25	....	. ..	....
5	0.26	1.34	1.35	1.34	1.40	1.21	0.15
pH (5 days)	4.8	5 5	5.2	5.7	5.8	6.2	5.8
Change in pH	+0.5	+0.3	−0.1	−0.4	−0.7	−0.8	−1.8
pH before sterilization	4.0	5.1	5.4	6.1	6.8	7.1	7.6

and below the above range tend to decrease yields. The changes in pH, during fermentation, show interesting trends; the highly acid media tending to become more alkaline while the highly alkaline media became more acid. All pH values tend to converge in the range of 5.1 to 5.8 with an average of 5.4. This phenomenon is receiving further study.

Since the pH values tend to converge at about 5.4, it was thought that the presence of an acid salt might increase the rate of oxidation. However, the data in table 5 show that while concentrations of primary potassium phosphate, up to 1 per cent, do not affect the final yields of ketose, the

TABLE 5. *Effect of concentration of KH_2PO_4 upon the yield of ketose*

Days	Grams KH_2PO_4 per 100 ml. of medium					
	0.000	0.100	0.300	0.600	0.800	1.000
1	0.33	0.32	0.20	0.16	0.11	0.06
2	1.38	1.31	1.16	0.84	0.67	0.52
5	1.37	1.37	1.32	1.40	1.40	1.40
7	1.40	1.42	1.42	1.42	1.42	1.42

initial rate of oxidation is markedly decreased by increasing concentrations of the salt. It is possible, however, that the phosphate ion itself played a part in the above phenomenon. The data in table 6 show that low concentrations of sodium chloride decrease both the rate of oxidation and final yield of the ketose.

TABLE 6. *Effect of concentration of NaCl upon yield of ketose*

Days	Grams NaCl per 100 ml. of medium				
	0.00	0.01	0.05	0.10	0.30
1	0.33	0.25	0.21	0.13	0.00
2	1.38	1.36	1.31	0.92	0.26
5	1.37	1.38	1.32	1.30	0.90
7	1.40	1.39	1.38	1.37	1.26

The rate of oxidation increases with increasing concentrations of yeast extract (table 7) but the final yields are not appreciably affected. While the highest yield of ketose is obtained with 5 per cent inositol (table 8) the percentage conversion is somewhat less than with 3 per cent of the substrate. At concentrations of inositol higher than 5 per cent both the actual and percentage yield are materially decreased.

The above results indicate the most desirable cultural conditions to be 3 per cent inositol, 0.05 per cent sorbitol, 0.5 per cent yeast extract, pH = 6.1, a surface volume ratio of 2.83 and an incubation temperature of 28°C.

EFFECT OF SUBSTRATES OTHER THAN SORBITOL

It was shown that small concentrations of sorbitol in the medium permit the indefinite subculture of the organism on an i-inositol medium. The following compounds were tested in substitution for the sorbitol: ascorbic acid, erythritol, dextrose, glycine, glycerol, mannitol, ethanol and the calcium salts of acetic, fumaric, glutaric and succinic acids. The

TABLE 7. *Effect of concentration of yeast extract upon yield of ketose*

Days	Grams yeast extract per 100 ml. of medium				
	0.10	0.30	0.40	0.50	1.00
1	0.19	0.25	0.28	0.33	0.45
2	0.75	1.11	1.34	1.38	1.48
5	1.36	1.33	1.31	1.37	1.42
7	1.36	1.38	1.36	1.40	1.46

substrates were employed at 0.5 per cent concentration. Only those media containing erythritol, dextrose, glycerol and mannitol exhibited growth and the oxidation of the i-inositol. Varying concentrations of the above substrates were then tested and the results were quite similar to those obtained by the use of sorbitol. Evidently all the substances studied which are readily dissimilated by the organism also serve the same role as does sorbitol in the fermentation of the i-inositol.

TABLE 8. *Effect of concentration of i-inositol upon the yield of ketose. Ketose expressed as grams per 100 grams of inositol*

Days	Grams inositol per 100 ml. of medium				
	1.00	3.00	5.00	7.00	10.0
1	25	11	4	1	0
2	46	46	18	5	9
5	46	46	37	16	9
7	46	46	43	24	11

EFFECT OF SORBITOL UPON SUBSTRATES OTHER THAN I-INOSITOL

Results in these laboratories confirmed the findings of others that *Acetobacter suboxydans* does not oxidize dulcitol, rhamnitol or rhamnose as sole substrates. Various concentrations of sorbitol were added to media containing varying amounts of the above compounds. In no case did the amount of reducing compound exceed that calculated as produced from the sorbitol. That is, while the presence of sorbitol in the medium permits the oxidation of i-inositol, it does not serve this function with dulcitol, rhamnitol or rhamnose.

Varying concentrations of sorbitol were added to media containing varying amounts of erythritol, glycerol or mannitol, substrates known to

be readily oxidized by the organism. In no case did the sorbitol stimulate the oxidation or alter the optimum concentrations of the three polyhydric alcohols.

In the early phases of this work it was thought that the action of the sorbitol might be associated with a metabolic product derived therefrom, that is, a type of growth factor permitting the organism to develop enzyme systems capable of oxidizing the inositol. However, experiments involving the addition of fermented sorbitol media showed the stimulation to be proportional to the amount of remaining unfermented sorbitol. Several gallons of molasses from submerged growth sorbose fermentations were kindly furnished by the Ames Station of the U. S. D. A. Agricultural By-products Laboratory. Any metabolic factor associated with the above phenomenon should be highly concentrated in this material. However, as found above, the stimulation was again proportional to the unfermented sorbitol. Sorbose had no stimulating effect upon the oxidation. Similar results were obtained with other substrates which could be substituted for the sorbitol in the inositol fermentation. These findings indicate that the adjuvant materials simply serve as sources of assimilable carbon permitting the organism to multiply and oxidize the inositol.

It is noteworthy that while certain chemicals permit the dehydrogenation of i-inositol they do not function similarly with dulcitol, rhamnitol or rhamnose. This apparent selectivity is receiving further study. Either the oxido-reductase system responsible for the dehydrogenation is transitory in nature, since the proliferating cell seems necessary for its functioning, or the system of reactions involved is a manifestation, of that "perfect harmony" which Kluyver (1931) says "is the exclusive prerogative of the living cell."

The polyhydric alcohols studied thus far in these laboratories fall into four classes in regard to the relation of concentration and yield of ketose by the action of *Actobacter suboxydans:*

I.   Those polyhydric alcohols dehydrogenated at high concentrations (25 per cent and above). These include sorbitol (2) and mannitol (3).

II.   Those polyhydric alcohols showing an optimum at a relatively low concentration. For example, glycerol (9) at 6 per cent and erythritol (10) at 4.5 per cent.

III.   Those polyhydric alcohols dehydrogenated in the presence of sorbitol or other source of assimilable carbon. For example, i-inositol.

IV.   Those polyhydric alcohols not dehydrogenated even in the presence of an assimilable source of carbon such as sorbitol. For example, dulcitol and rhamnitol.

SUMMARY

1.   *Acetobacter suboxydans* cannot be successfully subcultured on an i-inositol yeast extract medium. Sorbitol, in concentrations as low as 0.05 per cent, obviates this difficulty and permits the ready oxidation of the inositol. Erythritol, glycerol, dextrose and mannitol act as effectively as does sorbitol.

2. The addition of substrates effective in permitting the dehydrogenation of i-inositol do not permit the oxidation of dulcitol, rhamnitol or rhamnose.

3. The positive effect of the adjuvant materials in the inositol fermentation is not associated with metabolic products thereof. They simply serve as assimilable substrates permitting the growth of the organism and subsequent oxidation of the inositol.

4. The presence of sorbitol does not appreciably affect the dehydrogenation of other assimilable polyhydric alcohols.

5. Details are given for the development of cultural conditions optimum for the oxidation of i-inositol by means of *Acetobacter suboxydans*.

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ABSTRACTS OF DOCTORAL THESES[1]

CONTENTS

[1] Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

EVALUATION OF THE GERMICIDAL PROPERTIES OF SODIUM HYDROXIDE AND EFFECT OF ACIDITY ON GROWTH OF YEASTS[1]

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During the past few years national, state and local sanitary agencies have been active in setting up standards for mechanical bottle washing in beverage, dairy and other food industries. The standards proposed and established usually specify a minimum time of exposure of the bottles to a stipulated minimum concentration of alkali or caustic solution which is maintained at a designated minimum temperature. With few exceptions these standards are precise and inflexible since there has not been available a suitable method for converting a given standard as-to time of exposure, temperature and concentration of alkali, into one of equivalent sterilizing efficiency, if any of these factors was varied. Hence, a mathematical expression was sought which could be utilized to evaluate the germicidal properties of alkalies for various combinations of these three factors.

In addition to the foregoing investigations, the effect of acidity and nature of the substratum on the growth of yeasts which are responsible for 85 per cent of the economic loss from spoilage of carbonated beverages, was studied.

PART ONE

GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE

Solutions employed in mechanical bottle washers generally contain NaOH to which has been added milder alkalies. The germicidal properties of these mixtures as determined by various workers using *Bacillus metiens* have been found to depend primarily upon the amount of NaOH, but the proportion of milder alkali present also is significant. Since the spores of this organism were found not to die off logarithmically, that is, at a rate constantly proportional to the number of viable organisms present at any given time, the killing time (specifically, the time necessary to kill 99.9 per cent of the viable spores of *B. metiens*) was studied as a suitable measure of germicidal power.

The killing time for this organism was found to be a function of the temperature and caustic content of the disinfecting solution. The following equation was derived by the method of least squares from the available published data on disinfection of *B. metiens* with alkalies:

$$\log \theta = 4.9815 - 1.7912 \log C - 0.0563 \, t \qquad (1)$$

where θ is the killing time in minutes, C is the concentration of caustic in percentage by weight and t is the temperature in degrees centigrade. The error of estimate between the calculated and experimental killing times did not exceed 3.5 per cent.

Equation (1) when plotted in three coordinates generates a plane surface. The latter graph not only aids in clarifying the interrelationships among the three factors, killing time, temperature and concentration of caustic but also indicates how future experiments on killing times may be designed in order to obtain data of maximum statistical value.

The interpretations of the constant and coefficients in equation (1) are as follows: Let equation (1) be written:

$$\log \theta = K - a \log C - bt \qquad (2)$$

Phelps (1911) reported that concentration and temperature coefficients apparently do not vary with the test organism. In equation (2) a and b are associated with the effect of concentration and temperature, respectively, on the germicidal properties of the alkalies used and are independent of the test organism. In this connecton the effect of temperature alone on the spores of B. *metiens* was practically nil at 50°, 60° and 70° centigrade. For this reason b should be considered associated with the germicide and its reactivity at various temperatures.

On the other hand K in equation (2) is a constant characteristic for the resistance of the test organism against the test disinfectant. Thus for a given germicide a large value of K signifies that the organism is very resistant. Conversely a low value indicates little resistance. Moreover, when standards for bottle washing are stipulated by law there has in effect been established a standard of germicidal efficiency adequate to destroy a hypothetical organism the resistance of which is measured by the value of K in equation (2).

The foregoing explanations indicate possible uses for the constant and coefficients in equations (1) and (2).

In the first place a given standard may be accurately and conveniently converted into equivalents of time (exposure time), temperature and concentration of NaOH. Such equivalents have the same germicidal efficiency as the standard in question and may be used when a specific standard is difficult to meet either because of washing machine design or poor operation economy.

The equivalents of a standard may be determined by first substituting in equation (2) the time, concentration of NaOH, and the temperature stipulated by the standard being considered. Then solve for K using $a = 1.7912$ and $b = 0.0563$ ($b = 0.03129$ when temperature is in degrees Fahrenheit), the characteristic values when NaOH is employed as a germicide. Finally, having found K, it is possible to compute equivalents for various combinations of time, temperature and concentration.

In the above manner the equation for calculating the germicidal equivalents based on the New York City specifications for washing milk

bottles [$\theta = 7$, $C = 2$ per cent and $t = 65.56°C$. (150°F.)] was found to be:

$$\log \theta = 5.0753 - 1.7912 \log C - 0.0563 \, t \tag{3}$$

For the Chicago standard [$\theta = 5$, $C - 1.6$, $t = 48.89°C$. (120°)] to the equation for computing the germicidal equivalents is:

$$\log \theta = 3.8171 - 1.7912 \log C - 0.0563 \, t \tag{4}$$

In order to obtain the germicidal equivalents for the American Bottlers of Carbonated Beverages standard [$\theta = 5$, $t = 54.44°$ C. (130° F.)., $C = 3$ per cent alkali of which 1.8 per cent is caustic] for washing beverage bottles, it was necessary to mathematically evaluate the germicidal properties of solutions containing mixtures of NaOH and milder alkalies such as Na_2CO_3, Na_3PO_4, etc. Calculations based on data previously determined by Levine and his co-worders indicated that for a given temperature:

$$\log \theta = K - d \log C \tag{5}$$

where θ is the killing time in minutes, C, the total percentage of alkali calculated as NaOH, K, a constant depending on the temperature and the resistance of the organism and d, a coefficient dependent upon the composition of the germicide (alkali). When the solution was composed of NaOH and Na_2CO_3, d was found to be 0.6331. For solutions containing Na_3PO_4, d was somewhat greater (0.8308).

By using equations (2) and (5) in which $a = 1,7912$, $b = 0.0563$, $d = 0.6331$, an equation was derived from which germicidal equivalents for the American Bottlers of Carbonated Beverages standard may be computed. This equation is:

$$\log \theta = 4.3595 - 1.7912 \log C - 0.0563 \, t \tag{6}$$

As pointed out before, the constant (K) in the equations above is associated with the resistance of organisms subjected to killing. Associated as it is with the resistance of an organism, the value of K for different standards becomes a simple figure by which to compare bottle washing standards. For example, under the same conditions of temperature and concentration of washing compound how does the New York standard compare with the Chicago standard? By subtracting K Chicago = 3.8171 from K New York City = 5.0753 and taking the antilog, one obtains 18.1. This number means that the New York City standard for washing milk bottles is designed to kill a hypothetical organism, the killing time of which is 18.1 times greater that that of the hypothetical organism representing the Chicago standard. In similar manner the New York City standard organism required 5.2 times as long to kill as the A.B.C.B., which required 3.5 times as long as the Chicago standard organism.

Another way to compare washing solution standards is to compare them on the basis of concentration of caustic required to effect the same

germicidal killing efficiency at a given time and temperature. In order to do this, it is necessary to solve equations (3), and (4), and (6) for log C and then take the antilog of the difference between any two K values corresponding to the standards in question. On this basis the New York City organism required 5.02 times as much caustic as the Chicago organism for a given temperature and killing time. The A.B.C.B. organism required 2.5 times more caustic than the Chicago organism and New York City about twice as much as the A.B.C.B. hypothetical test organism employed as a standard.

PART TWO

EFFECT OF REACTION AND NATURE OF SUBSTRATUM ON GROWTH OF YEASTS

The studies on the effect of reaction (pH) and nature of substratum on the growth of yeasts isolated from carbonated beverages centered around the use of citric, lactic, and phosphoric acids and their salts. All of the test solutions contained a basal medium (5 grams dextrose, 3 grams peptone, and 5 grams salt mixture) plus a given amount of salt of one of the acids acidified with the corresponding acid, the amount of which was predetermined by electrometric titrations. In some instances cane sugar sufficient to make a 10 per cent solution was dissolved in the test solutions.

The yeast cultures employed in this phase of the work were selected from a number of strains isolated from contaminated or spoiled carbonated beverages. Selection was so accomplished that each yeast chosen represented a group of yeasts, each group being formed on the basis of morphology, spore formation, and certain cultural characteristics.

Of each of the selected strains 0.1 ml., containing about 200 cells of a properly diluted 48-hour glucose broth culture of each strain, was transferred to 10 ml. portions of the various test media and to petri dishes for plate counts of the inocula. Incubation of the media was at 27°C. for a period of not less than 8 days. All tests were made in duplicate or triplicate.

In general the addition of sodium phosphate to the medium decreased the acidity at which the yeast strains grew. The limiting H+ ion concentrations for the 17 strains of yeasts employed in a medium containing glucose, peptone, a salt mixture and in some cases 10 per cent cane sugar was about pH 2.0 to 2.5 when citric or phosphoric acids were employed. With lactic acid, however, the limiting H+ ion concentration was more alkaline—pH 2.5 to 3.0.

The addition of sodium phosphate or citrate to media acidified with phosphoric or citric acids respectively resulted in lowering the limiting hydrogen ion concentration at which yeasts grew by 0.5 to 1.0 pH unit. With lactic acid and its salts and effect was much greater. In many instances the yeasts which grew at pH 2.5 in media acidified with lactic acid did not grow in media the reaction of which was less than pH 3.0

when 0.1 N sodium lactate was present and less than pH 3.5 when 0.2 N sodium lactate was present. In some instances the yeasts which grew at pH 3.0 in the absence of the sodium salt of lactic acid failed to grow at reactions as alkaline as pH 4.3 when 0.2 N or 0.4 N sodium lactate was present in the medium.

The limiting acidities in the presence of 10 per cent sugar were in general the same as in the absence of cane sugar, although in certain instances yeasts were unable to grow at acidities as acid as when the sugar was not included in the test media. In several instances greater numbers of organisms had to be inoculated into the test media containing sugar in order to initiate growth.

SUMMARY

1. The interrelationships between killing time, concentration of NaOH and temperature in evaluating the germicidal efficiency of caustic solutions when killing B. metiens spores are expressed mathematically by an equation of the following order:

$$\log \theta = K - a \log C - bt$$

2. The above equation may be utilized in calculating germicidal equivalents for stipulated standards and for accurately comparing various standards for bottle washing.

3. The limiting H-ion concentration for growth of yeasts in media acidified with phosphoric, lactic or citric acids was lowered by addition of their respective sodium salts.

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I. HALOGEN AS A FINISH FOR WOOL
II. FORMALDEHYDE AS A FINISH FOR WOOL
III. COMPARISON OF THREE TEXTILE DETERGENTS[1]

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The stability of the modified keratins, chlorinated wool and formaldehyde-treated wool, to acid and alkali and the stability of cotton, regenerated-cellulose rayon, cellulose-acetate rayon, silk, wild silk, and wool to repeated washings with soap, silicated soap, and sulfated alcohol have been measured by changes in the composition, weight, and mechanical properties of these textile fibers.

I. HALOGEN AS A FINISH FOR WOOL

Degradation of plain-woven wool, chlorinated by 0.06N hypochlorous acid in one hour at 25°C., by 0.50 to 6.00N hydrochloric acid in ten hours at 25°C., 0.25 to 0.75N hydrochloric acid in one hour at 100°C., and 0.05 to 0.20N sodium hydroxide in ten hours at 40°C., has been measured by the weight, nitrogen, sulfur, and wet strength of the residual wool.

Degradation of chlorinated wool by acid in ten hours at 25°C. exceeded that of wool as measured by sulfur or wet strength; the loss in weight and nitrogen was the same for wool and chlorinated wool at 25°C.

Both wool and chlorinated wool were much more degraded by acid at 100°C. than at 25°C., the chlorinated wool more than the wool, although chlorinated wool's ratio of sulfur to nitrogen was not decreased at either temerature.

Sodium hydroxide in ten hours at 40°C. dissolved the same amount of sulfur from chlorinated wool as from wool but lowered the nitrogen and weight of the chlorinated wool more. The ratio of sulfur to nitrogen of chlorinated wool decreased with increasing concentration of alkali. The wet strength of chlorinated wool was completely destroyed in one hour at 40°C. by 0.05N sodium hydroxide, although wool retained five per cent of its strength after ten hours at 40°C. in 0.20N sodium hydroxide.

II. FORMALDEHYDE AS A FINISH FOR WOOL

Degradation of plain-woven wool, treated with one percent formaldehyde for one hour at 70°C., by 6.00N hydrochloric acid in ten hours at 25°C., 0.25 to 0.75N hydrochloric acid in one hour at 100°C., 0.05 to 0.40N sodium hydroxide in ten hours at 40°C., and 38 per cent sodium

hydroxide for five minutes at 15°C., has been measured by the weight, nitrogen, sulfur, and wet strength of the residual wool.

Decrease in weight and strength was less for formaldehyde-treated wool than for wool in 6.00N hydrochloric acid at 25°C.

Formaldehye protected wool from loss in weight, nitrogen, sulfur, and wet strength when treated with acid at 100°C.; formaldehyde-treated wool retained 25 per cent of its wet strength after one hour at 100°C. in 0.50N hydrochloric acid although wool showed no measurable wet strength when so treated; upon treatment with acid the ratio of sulfur to nitrogen in the residual formaldehyde-treated wool remained constant.

Formaldehyde protected wool from loss of weight and strength when treated with dilute alkali for ten hours at 40°C.; the wet strength of wool was completely destroyed by 0.10N sodium hydroxide. Total nitrogen of the residual formaldehyde-treated wool remained constant; loss of nonsulfate sulfur by formaldehyde-treated wool coincided with that of wool at 0.05 and 0.10N alkali but was less at greater concentrations; the ratio of sulfur to nitrogen in the residual formaldehyde-treated wool decreased with increasing concentration of alkali.

The loss of sulfur, weight, and wet strength during five minutes at 15°C. in 38 per cent sodium hydroxide was the same for the formaldehyde-treated wool as for wool; total nitrogen of the formaldehyde-treated wool was unchanged by this treatment.

III. COMPARISON OF THREE TEXTILE DETERGENTS

Plain-woven cotton, regenerated-cellulose rayon; cellulose-acetate rayon, silk, wild silk, and wool have been washed separately by hand in 0.5 per cent solutions of neutral olive-oil soap, silicated soap, or sulfated alcohol for five minutes at room temperature, rinsed until the rinse no longer foamed, and dried in air and diffused light at room temperature. After ten, twenty, thirty, forty, and fifty washings all the fabrics were again analyzed for ash, percentage of light absorbed, shrinkage, wet warp breaking strength, elongation at breaking load, and weight. the silks and wool were analyzed for total nitroen and sulfate sulphur, the wool for total sulfur, and the cellulose acetate for acetyl.

The acetyl value of the cellulose-acetate rayon increased by 0.5 per cent in ten washings but then remained constant.

With each of the detergents the ash of the cellulosic fabrics decreased and that of the proteic fabrics increased with increasing number of washings. The increase in ash for wild silk and wool was greatest with silicated soap and least for wool with sulfated alcohol.

Total nitrogen of the residual wild silks reflected the loss of non-nitrogenous sizing during washing, that of silk and wool the increased weight of the residual fabrics.

Repeated washing bleached cellulose, and this effect was greatest with silicated soap; silk, wild silk, and wool darkened upon repeated washing with sulfated alcohol.

Fabrics other than wool shrank but little in any of the detergents.
The wet strength of all the fabrics decreased with increasing number of washings in each of the three detergents. In fifty washings the wet strength of silk decreased 76 per cent with sulfated alcohol, 10 per cent with silicated soap, and 24 per cent with soap. Wild silk lost 74 per cent of its wet strength with sulfated alcohol and 78 per cent with silicated soap in fifty washings; wool lost 11 per cent of its wet strength with sulfated alcohol, 19 per cent with silicated soap, and 21 per cent with soap in fifty washings.

The residual sulfur of wool washed with silicated soap or soap remained constant but that of wool washed with sulfated alcohol increased; silk and wild silk also retained sulfate sulfur from the sulfated alcohol.

The relatively great losses in weight of the cotton and wild-silk fabrics upon washing are explained by their loss of sizing; regenerated cellulose, contrasted with cellulose acetate, gained slightly in weight upon washing; wool gained more in weight than silk with each of the three detergents.

DEFECTS OF BLUE (ROQUEFORT TYPE) CHEESE[1]

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Since the ripening of the different cheeses is primarily a biological process, variations in the results are to be expected. In blue cheese, as in most other types, some of the variations are of a minor nature and the cheese showing them are still considered satisfactory. In other cases the variations are of more importance, the cheese involved being definitely defective.

The objectionable conditions encountered in blue cheese vary widely, and some of them are difficult to classify. However, a number of rather specific defects have been noted often enough to make them of considerable practical importance. In general, the specific defects of blue cheese are essentially the same as those encountered in other cheeses.

An improvement in the general quality of blue cheese requires a reduction in the number of cheese showing minor variations from the most desirable qualities and the elimination of the cheese showing definitely objectionable conditions. As a basis for this, the causes of the various defects must be etablished.

In the work reported, an attempt was made to determine the causes of a number of the more serious defects of blue cheese.

A defect of blue cheese in which a portion of the edges became soft appeared to be caused by excessive moisture in the softened part of the cheese. The logical place for softening to occur would be the edge, since here two surfaces are separated by a relatively small amount of cheese and the ratio of desposited moisture to cheese is high. The comparative analyses of several normal and several defective cheese showed that the moisture content of the soft material was from 8.19 to 16.91 per cent higher than the moisture content of the normal cheese. The defect was readily reproduced by placing cheese near a humidifier where free moisture could strike it.

Gas producing organisms are found in various dairy products, including cheese. The most common gas producing organisms causing defects in cheese are members of the Eschericia-Aerobacter group. Gas formation is of relatively little importance in blue cheese, presumably because of the open texture, which permits the gas to escape, and the unfavorable conditions in the cheese for growth of the common gas forming organisms. Trials with a culture of *Aerobacter aerogenes* freshly isolated from gassy cheddar cheese showed that inoculations

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[1] Original thesis submitted June, 1940. Doctoral thesis number 567.

(of the milk) which resulted in very gassy cheddar cheese caused no gas holes or only insignificant numbers in blue cheese.

A lack of mold growth occasionally is noted in blue cheese. In certain cases it probably is caused by too short a ripening period. With further ripening such cheese commonly develops a normal mold growth and flavor. In other cases the cheese lacks mold growth even after extended ripening. A defect of blue cheese in which the blue mold failed to develop in the cheese, even after extended ripening, was apparently caused by the use of a mold powder in which an atypical strain of *Penicillium roqueforti* predominated. The variation in the mold may have been caused by the long continued cultivation on an artificial medium of the culture used to prepare the powder. In order to avoid slow mold development in the cheese, the mold powder must be carefully controlled. It is advisable to establish the effectiveness of a new lot of mold powder by using it in one or two runs of cheese before it is employed in more extensive operations. There may be an advantage in occasionally isolating a fresh culture of *Penicillium roqueforti* from a good blue cheese and using it in the preparation of mold powder.

A fruity flavor in blue cheese seemed to be associated with a relatively high moisture content. No organism capable of reproducing the defect could be isolated from the defective cheese, and a yellow green mold present in some of the defective samples did not cause fruitiness in cheese, although it produced a fruity, yeast like odor in milk. A relatively high moisture content in cheese may so influence the activity of the normal blue cheese organisms that the growth products deviate somewhat from the usual type.

Blue cheese is characterized not only by its flavor but also by the blue veins through it. The veins are due to the growth of *Penicillium roqueforti* and are expected in a normal cheese. Molds other than *Penicillium roqueforti* occasionally invade blue cheese. If the color produced by them is rather similar to that of the normal blue portions, it is overlooked, but if it is not similar, its presence is immediately noticed and it is designated as a color defect. One of the most noticeable of these color defects is a black discoloration. This black discoloration is usually accompanied by a musty flavor and was attributed to the growth of *Hormodendrum olivaceum* in the cheese.

A defect in which a gray discoloration and a mousy, ammoniacal flavor developed in blue cheese was accompanied by an increase in pH. In every case the pH of the discolored cheese was higher than that of the corresponding normal cheese and in most cases the difference was significant. The variation from the normal ripening mechanism which caused the gray discoloration presumably involved the formation of basic products from protein. Extensive development of *Penicillium roqueforti* or growth of contaminating organisms could be responsible for an unusual protein decomposition. Contaminating organisms capable of reproducing the defect could not be isolated.

MEASUREMENT OF THE HEAT LIBERATED IN THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS[1]

RALPH E. CARLYLE

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The phenomenon of heat evolution by moist plant materials has been the subject of much speculation and study. This process, sometimes known as thermogenesis, has attracted many investigators not only because of its theoretical importance but also because of its many practical ramifications. Thermogenic fermentations raise some fascinating biological problems, since the rapid change of temperature must profoundly affect the nature of the active microbial population. The effect of plant composition has hardly been examined, and it remains to be determined whether the evolution of heat is associated with the rapid fermentation of some particularly available or thermogenic constituent. Furthermore, up to the present no attempt has been made to determine quantitatively the heat evolved in such fermentations.

In this investigation the construction and operation of an apparatus for study of this general problem are described along with the results of some thermogenic decompositions using oat straw as a substrate. The apparatus designed to measure heat evolution quantitatively consists essentially of a fermentation vessel immersed in a well inoculated cylindrical water bath, the temperature of which is both controlled by and remained the same as the fermenting material in the jar. In this way an adiabatic system is obtained with no heat loss or gain during the fermentation. The fermentation vessel is a glass quart fruit jar. Over the jar is fitted a flanged cover, made of brass with four threaded openings into which are screwed four sleeves, two small and two slightly larger. The smaller openings accommodate the glass air inlet and air outlet, the former reaching to the bottom of the vessel. One of the two larger openings accommodates a thermocouple and the other a two-junction thermopile, both being held in place by small rubber stoppers which act as air and water tight gaskets and each extending into the center of the plant material in the vessel. The cover is fastened over the top of the glass jar by means of a clamp, a rubber gasket being used to prevent leaks of air or water.

The temperature of the bath is controlled by two 2-junction thermopiles, one each in flask and bath respectively, connected directly to a galvanometer, the reflected beam from the mirror of which is focused on a photo-electric cell. Any deflection of the beam caused by an increase in temperature in the flask causes a relay to switch the heaters on and off several times a minute when heat evolution was rapid, so that no

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[1] Original thesis submitted June, 1940. Doctoral thesis number 561.

temperature differences between the flask and the bath could ordinarily be detected.

Copper-constantan thermocouples, a pH potentiometer and a galvanometer are used in making the temperature measurements. The measuring system is calibrated against an accurate thermometer and a detailed curve drawn for the desired temperature range so that potentiometer readings can be converted directly into degrees centrigrade. Aeration of the flask is carried out by passing air (at a rate of about 16 liters per day) from a compressed air cylinder through a long copper coil in the bath and saturating it at that temperature by passage through a wet bead tower also immersed in the bath. The evolution of CO_2 may be determined from hour to hour, and this provides additional information as to the rate of fermentation.

The procedure in using the apparatus was as follows: The fermentation was started at a temperature in the neighborhood of 25°. Forty grams of cut oat straw was moistened with water containing 1.3 grams of ammonium nitrate. No inoculation was ordinarily made, unless the straw had been given a chemical pre-treatment. The lid was sealed tightly and aeration commenced. The circuit controlling the heaters was swtiched on when the straw in the fermentation vessel had come into equilibrium with the water bath. As the fermentation proceeded, temperature readings and CO_2 determinations were made at frequent intervals. When the temperature had ceased to rise the fermentation vessel was removed from the bath and the loss of dry matter ascertained by weighing the wet residue and determining the moisture content in duplicate. In some cases plate counts were made of bacteria in the wet residue.

Using the adiabatic apparatus and a cut oat straw substrate, experiments were made on the following phases of the subject:

(a) Rate of heat and CO_2 evolution
(b) Substrate exhaustion
(c) Modification of the substrate by extraction
(d) Nitrogen requirements of the microbial population
(e) Bacterial numbers at different stages
(f) Pure culture studies using an unidentified bacillus, and *Aspergillus fumigatus*

The results and conclusions of the investigation are as follows:

1. Temperature increases of 50°C. were obtained in less than 48 hours. Heat evolution rate curves showed two maxima, one in the mesophilic range followed by one in the thermophilic range. The extent of decomposition usually found in one temperature ascent was less than 4 per cent on an oven dry basis. The rate of heat evolution was the important factor rather than the total amount of heat evolved. Rates of heat and carbon dioxide evolution were closely related.

2. Heat evolution was expressed quantitatively in terms of calories. In a typical fermentation in which the temperature increased from 25.8°

to 69.8°C., it was calculated that 5894 calories of heat were evolved. This value cannot be considered absolute but is a close approximation.

3. As the readily available plant constituents were decomposed, the rate of heat evolution decreased. Adding additional water-soluble plant material resulted in the phenomenal maximum heat evolution rate of 4.9° per hour. The polyuronide hemicelluloses proved to be available for purposes of heat evolution, but ordinarily only the water-soluble constituents were involved in the decomposition accompanying one temperature ascent.

4. The treatment of straw with hot water, 0.5 per cent NaOH, 0.5 per cent H_2SO_4 and 3.0 per cent H_2SO_4 resulted in material decreases in rate of heat evolution, the decreases being in the order named.

5. Additions of a soluble nitrogen source proved of no benefit to decompositions in which only one temperature ascent occurred.

6. Bacterial numbers and rate of heat evolution appeared to be closely related, especially in the mesophilic temperature range. The relationship was not evident in the thermophilic temperature region, probably because the count plates were not incubated at thermophilic temperatures.

7. *Aspergillus fumigatus* and an unidentified bacillus in pure culture fermentations exhibited activity only in the mesophilic range.

SOME PREFERENTIAL REACTIONS OF POLYFUNCTIONAL COMPOUNDS[1]

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This work was undertaken in order to determine the usefulness of several moderately reactive organometallic types in effecting preferential reactions with groups in polyfunctional compounds. The study includes reactions of organomagnesium and organolithium compounds with cyano esters, ketonic esters and ketonic nitriles and reactions of organo-cadmium compounds with ester acid chlorides, ketonic acid chlorides and diacid chlorides.

In all the experiments the organometallic compound was added dropwise to a rapidly-stirred solution of the difunctional compound contained in the customary apparatus[2] filled with purified nitrogen.

The yields of reaction products, calculated on several different bases, are given together and designated as follows:

(1) %' indicates the yield based on one-half of the difunctional compound added.

(2) %" indicates the yield based on all the difunctional compound added.

(3) % indicates the yield based on the actual amount of difunctional compound used up.

Five-hundredths mole of methyl p-cyanobenzoate and 0.05 mole of methylmagnesium iodide gave a 38%', 40% yield of 4-(2-hydroxy-2-propyl) benzonitrile based on the corresponding acid isolated on hydrolysis. The amount of cyano ester recovered was 53%. When two equivalents of Grignard reagent were used, the yield of the hydroxy acid was 40%", 54% with a 26% recovery of cyano ester. Two equivalents of phenylmagnesium bromide and the cyano ester produced a 54%" yield of *(p-cyanophenyl)diphenylcarbinol*, m.p. 91-92°. Equimolecular amounts of the reactants gave the hydroxycarbinol (55%'), p-benzoylbenzonitrile (1.4%") and unreacted ester (33%).

From 0.1 mole of methyllithium and 0.05 mole of methyl p-cyanobenzoate there was obtained a 53%", 61% yield of 4-(2-hydroxy-2-propyl) benzonitrile based on the corresponding hydroxy acid isolated. A 14% recovery of ester was realized. Equimolecular portions of phenyllithium and cyano ester gave (p-cyanophenyl)diphenylcarbinol (13%'), (p-benzoylphenyl)diphenylcarbinol (6.6% based on RM used), and cyano ester (30% recovery). In a second experiment the yields of products were 9.8%', 9.5% and 34%, respectively.

[1] Original thesis submitted December, 1939. Doctoral thesis 547.
[2] Gilman, Zoellner and Selby, *J. Am. Chem. Soc.*, 54, 1957 (1932).

Two equivalents of phenylmagnesium bromide with methyl m-cyano-benzoate produced a 46%″ yield of (m-cyanophenyl)diphenylcarbinol (based on the *(m-carboxyphenyl)diphenylcarbinol*, m.p. 163°, obtained, which was characterized as *(m-carbomethoxyphenyl)diphenylcarbinol*, m.p. 140°) and a 2.9% (based on RMgX) yield of *(m-benzoylphenyl)-diphenylcarbinol*, m.p. 126°. In a second experiment there was isolated *(m-cyanophenyl)diphenylcarbinol*, m.p. 96°, (13%″) and its correspond-ing acid (32%″) which represented a total yield of 45%″ for the cyano-carbinol.

Equimolecular amounts of methyl p-benzoylbenzoate and methyl-magnesium bromide gave, after hydrolysis by alkali, a 77%″ yield of *4-(α-hydroxy-α-phenylethyl)benzoic acid*, m.p. 145-146°, [characterized by the methyl ester, *methyl 4-(α-phenylethynl) benzoate*, m.p. 73.5-74°]. From phenylmagnesium bromide under similar conditions, there re-sulted (p-carbomethoxyphenyl)diphenylcarbinol (56.5%″, 66%″ crude).

Methyllithium and the keto ester produced a 45%‴, 55% yield of the carbomethoxycarbinol when based on the amount of 4-(α-hydroxy-α-phenylethyl)benzoic acid isolated. On the other hand phenyllithium and the ketonic ester gave a 51% yield of 1,4-di-(diphenylhydroxymethyl)benzene (based on RM used).

Equivalent amounts of methyl p-acetylbenzoate and methylmag-nesium bromide gave a 53.4%″, 64% yield of methyl 4-(2-hydroxy-2-propyl)benzoate based on the amount of 4-isopropenylbenzoic acid iso-lated. A second experiment gave a 64%″ yield of the unsaturated acid. Phenylmagnesium bromide and the keto ester produced in one run a 45%″ yield of methyl 4(α-phenylethenyl)benzoate (from dehydration of hydroxy ester) and in a second run a 57%″ yield of methyl 4(α-hydroxy-α-phenylethyl)benzoate based on the corresponding acid isolated.

From equivalent amounts of methyl p-acetylbenzoate and methyl-lithium there was isolated a 7.7% yield (based on RM) of di-[4-(2-hydroxy-2-propyl)]benzene. An excess of methyllithium gave the dihy-droxy compound (53%) and 4-(2-hydroxy-2-propyl)acetophenone (based on the semicarbazone). Phenyllithium and the keto ester (equivalent amounts) produced [4-*(α-hydroxy-α-phenylethyl)phenyl]diphenylcarbi-nol*, m.p. 138-139°, and methyl 4-(α-hydroxy-α-phenylethyl)benzoate (8%″, 16%, based on the hydroxy acid isolated).

p-Benzoylbenzonitrile with methyl- and phenylmagnesium bromides gave an 84%″ yield of 4-(α-hydroxy-α-phenylethyl)benzonitrile and a 67%″ yield of (p-cyanophenyl)diphenylcarbinol, respectively. From methyllithium there were obtained a 45%″ yield of 4-(α-hydroxy-α-phenylethyl)benzonitrile and a 6.7% yield of 4-(α-hydroxy-α-phenyl-ethyl)acetophenone (isolated only as its *semicarbazone*, m.p. 182-183°). Phenyllithium, on the other hand, gave a 58%′ yield of (p-benzoyl-phenyl)diphenylcarbinol (yield 72%′ on the basis of the ketimine hydro-chloride of the hydroxy ketone) and a 16%″ yield of (p-cyanophenyl)-diphenylcarbinol.

p-Acetylbenzonitrile and methyl- and phenylmagnesium bromides gave 4-(2-hydroxy-2-propyl)benzonitrile (54%" on basis of corresponding acid) and a 69%" yield of *4-(α-hydroxy-α-phenylethyl)benzonitrile*, m.p. 91-92°, respectively. Methyllithium and the keto nitrile formed only 4-(2-hydroxy-2-propyl)acetophenone (37%' on the basis of its *semicarbazone*, m.p. 213°). Phenyllithium yielded *4-(α-hydroxy-α-phenylethyl)-benzophenone*, m.p. 106°, (22%') [*oxime*, m.p. 140°] and 4-(α-hydroxy-α-phenylethyl)benzonitrile (23%", 34%).

From equivalent amounts of p-benzoylbenzoyl chloride and phenylmagnesium bromide there was isolated p-dibenzoylbenzene (12.6%", 16%) and (p-carboxyphenyl)diphenylcarbinol (4.7%", 6%). A second run gave a 15%", 18% yield of the diketone and a 5.5% yield of (p-benzoylphenyl)diphenylcarbinol. An excess of diphenylcadmium and the acid chloride yielded p-dibenzoylbenzene (51%").

Terephthalyl chloride (1 mole) and dimethylcadmium (0.5 mole) produced p-acetylbenzoic acid (4%") and crude diacetylbenzene (33%"). Diphenylcadmium and the acid halide gave p-dibenzoylbenzene (34%', 43%), and p-benzoylbenzoic acid (1.4%", 3.7%). Phthalyl chloride (0.1 mole) and diphenylcadmium (0.05 mole) formed 3,3-diphenylphthalide (49%').

One-tenth mole of sebacyl chloride and 0.05 mole of dimethyl-, di-*n*-hexyl- and diphenylcadmium gave 1,8-diacetyloctane (88%', 77%), *1,8-di-n-heptoyloctane*, (49%', 43%), m.p. 88°, [*disemicarbazone*, m.p. 166°], and 1,8-dibenzoyloctane (71%', 78%), respectively. From 9-carbethoxynonanoyl chloride and the same cadn.ium compounds, used in the same ratio, there were obtained diketones and keto acids. Dimethylcadmium formed 1,8-diacetyloctane (9.6%") and 10-ketoundecylic acid (22%", 38%); di-*n*-hexylcadmium produced 1,8-di-*n*-heptoyloctane (7%") and 10-ketopalmitic acid (40%". 54%); diphenylcadmium gave 1,8-dibenzoyloctane (17.5%") and 9-benzoylnonanoic acid (41%", 52%). When the acid chloride ester was added to an excess of the organocadmium compound the yield of diketone was increased slightly and the yield of keto acid was decreased about 5%.

Equivalent amounts of cinnamoyl chloride and phenylzinc chloride gave a 17.9%" yield of β-phenyl-γ-benzoyl-γ-benzohydrylbutyrophenone. Two moles of phenylzinc chloride gave a 34%" yield of the above product and a 4.8%" yield of benzalacetophenone. When the acid chloride was treated with an excess of diphenylzinc, a 35%" yield of β,β-diphenylpropiophenone resulted. Two moles of phenyllithium and the acid chloride yielded diphenylstyrylcarbinol (64%) and β,β-diphenylpropiophenone (6.6%), while phenylmagnesium bromide under the same conditions gave 1,1,5,5-tetraphenylpentanone-3 (50%') and β,β-diphenylpropionic acid (8%").

A correlation of the results obtained in this investigation shows that the Grignard reagent does react preferentially with one of the groups in the disubstituted benzene derivatives studied, and that the group which

reacts is the one which would have been expected on the basis of the series of relative reactivities of the various functional groups[2]. The organolithium compounds, on the other hand, tend to react with both groups and are too reactive to be used for preferential reactions. Organocadmium compounds are suitable reagents for the preparation of ketonic esters from acid chloride esters if the latter are pure and contain no diacid chlorides. Organocadmium compounds do not give ketonic acids in any appreciable yields with diacid chlorides, but give good yields of diketones. The results of the reactions of the various organometallic compounds with cinnamoyl chloride tend to indicate that the organozinc compounds react first with the unsaturated acid chloride by metathesis; that phenylmagnesium bromide adds 1,4 to the conjugated system of the acid chloride; and that phenyllithium adds first to the carbonyl group.

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[2] Entemann and Johnson, *ibid*, 55, 2900 (1933); Gooch, Master's thesis, Library, Iowa State College, 1939.

THE DEVELOPMENT OF WESTERN WHEAT STEM SAWFLY (CEPHUS CINCTUS NORT.) IN VARIOUS HOST PLANTS AS AN INDEX OF RESISTANCE[1]

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The western wheat stem sawfly (*Cephus cinctus* Nort.) is a pest of primary importance, which causes serious economic loss to wheat farming throughout the great plains of Western Canada. This insect is native and attacks a number of the small grains and grasses. During the past few years changes in farming practices have been introduced to control soil erosion, drought, and plant diseases. Although these changes have in themselves been beneficial they have made the control of *C. cinctus* much more difficult.

Certain varieties of wheat which have a tendency to produce pith filled culms, commonly designated as solid stemmed, have been found to be resistant in varying degrees to the attack of the wheat stem sawfly.

In 1933 a cooperative project, between the Cereal Division of the Experimental Station, Swift Current, Saskatchewan, and the Entomological Laboratory, Lethbridge, Alberta, was started for the purpose of investigating the possibility of producing a commercially desirable wheat that would also be resistant to wheat stem sawfly.

In order to get a more complete knowledge of the nature of resistance and its effect upon the insect itself a study of the development of *C. cinctus* in some of its host plants was started in 1937. This study was principally concerned with comparing and contrasting the growth and development of this insect in favorable hosts and those which were partially or wholly resistant.

Marquis and several other commonly grown *vulgare* spring wheats appear to be more suitable host plants than do the native grasses within which the sawfly developed before the introduction of wheat. These wheats were used as standards of comparison. Golden Ball, S615, S632, S633, and S493 have been studied as varieties of those wheats which are solid stemmed and thus show varying degrees of resistance. *Agropyron smithii, A. elongatum, A. cristatus, A. pauciflorum, Bromus inermis, Stipa viridula, Phleum pratense* and *Elymus dahuricus* were the cultivated and native grasses studied.

The indices of development which were used in this study were as follows:

(1) Larval head width
(2) Larval mortality
(3) Length of adult female
(4) Oviposition potential.

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[1] Original thesis submitted March, 1940. Doctoral thesis number 553.

The entire larval period is spent within the lumen of the host stem. It was therefore impossible to make periodic observations of individual larvae. Collections of larvae were made from time to time throughout the developmental period. The head capsules of these were measured and an attempt was made to determine growth rates from these data. Histograms of head widths were prepared to determine if possible the number of instars.

In Marquis wheat the number of instars as determined by "Dyar's Rule" appears to be five. In others the distinction is not easily made but appears to be variable. The variations are dependent upon many conditions. Evidently the succulence of the stem which is influenced by drought and the ripening process and the prevalence of pith are of prime importance in determining development.

On the whole the variability was so great that the interpretation of growth rates as based on the instars was abandoned and the mean width of all larvae was plotted against time. In this way it was possible to compare and contrast the rate of growth. With respect to the adults it was found that there is a linear relationship between the length of the female adult and the oviposition potential.

Those hosts in which the mature larvae were small produced small adults.

The pith tissue characterizing the solid stemmed varieties of wheat is affected in its expression by environment. Golden Ball retains its solidness under all conditions studied, although its consistency is variable.

Those varieties designated by "S" are less stable in their pith consistency in that they are completely hollow when grown in the greenhouse, and hollow to solid in a screen cage. There is a direct relationship between the amount and consistency of the pith tissue present and the degree of the effect upon the developing *C. cinctus*. It therefore appears as though the resistance is of a mechanical nature rather than a nutritional one.

The moisture content of the plant or its succulence may influence to some extent the length of the developmental period. Late maturing wheat varieties tend to produce larger larvae and adults. This condition is not necessarily an index of host suitability since the mortality in such stems is usually high.

Oviposition in stems is limited by the stage of development of the plant and the diameter of its stem.

Agropyron elongatum, an introduced grass which is crossable with wheat, is highly resistant. The culms of this species are largely solid although those which are predominantly hollow appear to have a deleterious effect upon larval development to such an extent that it is doubtful that any will survive and reach maturity.

Phleum pratense appeared to show a type of resistance entirely different from that of wheats and other grasses. The bulb-like basal portion of the stem is tunnelled by the larvae in preparing their hibernaculum.

During spring renegeration of the plant, the larvae are apparently crushed by the growth and enlargement of the surrounding tissue.

Varieties of oats appear to be entirely resistant to the wheat stem sawfly and from the data thus far available they appear to be deficient in some nutritional essential to the growth and development of this insect.

FLEAS OF EASTERN UNITED STATES[1]

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From the Department of Zoology and Entomology, Iowa State College

This is the first comprehensive revision dealing with American fleas since the appearance of Baker's[2] Revision of American Siphonaptera in 1904, despite the recognized importance of fleas as transmitters of bubonic plague and endemic typhus fever, and the growing concern about their implication in the transmission of some other diseases. Workers have described many new species in the ensuing 36 years, and new characters have been discovered which have led to a complete rearrangement of classification. These new species and characters were dealt with at the time of their discovery only in numerous short papers, most of which were published in various technical journals.

In this study, the author has drawn together, coordinated, and brought up to date these scattered bits of work. In addition, he has contributed the findings of his own original research carried on for more than three years at the United States National Museum and at the Iowa State College. Specifically, this study contains . . .

Descriptions of the distinguishing characteristics of 55 species, falling into 5 families comprising 33 genera, which are known to parasitize more than 75 mammalian and avian hosts including man and domestic animals in Eastern United States.

One hundred sixty-six figures, an average of 3 for each species, which illustrate the distinguishing characteristics, thus further facilitating easy and accurate identification. All illustrations and descriptions are original except a few which, because of the inability to obtain material, were adapted from previous publications.

Specifications of the known distribution, the known hosts, the type host, and the locality of occurrence for each of the 55 species.

Keys to the suborders of Siphonaptera, to the families of Integricipita and Fracticipita, and to the genera of the five families.

A host index in which hosts are listed alphabetically, and the fleas that parasitize each host are specified.

A synonymic index in which synonyms are listed alphabetically.

A selected bibliography of other published material dealing with fleas.

Brief chapters on collection and preservation of fleas, life history and control, and morphology and terminology.

Five families of fleas occur in the United States east of the one hundredth meridian—Hectopsyllidae, Pulicidae, Dolichopsyllidae, Hystrichopsyllidae and Ischnopsyllidae.

[1] Original thesis submitted April, 1940. Doctoral thesis number 555.
[2] Baker, U. S. National Museum Proceedings, 27:365–469 (1904).

The family Hectopsyllidae contains but one eastern species, *Echidnophaga gallinacea* (Westwood).

The family Pulicidae contains the following eastern species: *Hoplopsyllus affinis* (Baker), *H. lynx* (Baker), *Xenopsyllus cheopis* (Rothschild), *Pulex irritans* Linnaeus, *Cediopsylla simplex* (Baker), *Ctenocephalides felis* (Bouche), and *C. canis* (Curtis).

The family Dolichopsyllidae contains the following eastern species: *Rhopalopsyllus gwyni* C. Fox, *Trichopsylla floridensis* I. Fox, *T. lotoris* Stewart, *Ctenophthalmus pseudagyrtes* Baker, *Rectofrontia fraterna* (Baker), *Foxella ignota* (Baker), *Conorhinopsylla stanfordi* Stewart, *Ctenophthalmus pseudagyrtes* Baker, *Rectofrontia fraterna* (Baker), *Foxella ignota* (Baker), *Conorhinopsylla stanfordi* Stewart, *Opisocrostis bruneri* (Baker), *Oropsylla arctomys* (Baker), *Odontopsyllus multispinosus* Baker, *Ceratophyllus celsus* Jordan, *C. diffinis* J., *C. idius* Jordan and Rothschild, *C. gallinae* (Schrank), *C. niger* C. Fox, *C. riparius* Jordan and Rothschild, *C. swansoni* Liu, *Opisodasys pseudarctomys* (Baker), *Orchopeas wickhami* Baker, *O. caedens* (Jordan), *O. sexdentatus pennsylvanicus* (Jordan), *O. leucopus* (Baker), *Megabothris asio* (Baker), *M. acerbus* (Jordan), *M. quirini* (Rothschild), *M. wagneri* (Baker), *M. vison* (Baker), *Mosopsyllus fasciatus* (Bosc).

The family Hystrichopsyllidae includes the following eastern species: *Hystrichopsylla gigas tahavuana* (Jordan), *Atyphloceras bishopi* Jordan, *Stenoponia americana* (Baker), *Peromyscopsylla hesperomys* (Baker), *P. scotti* I. Fox, *P. catatina* (Jordan), *Ctenopsyllus segnis* (Schönherr), *Nearctopsylla genalis* (Baker), *Doratopsylla blarinae* C. Fox, *D. curvata* Rothschild, *Epitedia wenmanni* (Rothschild), *E. faceta* (Rothschild), *E. testor* (Roths.), *Tamiophila grandis* (Rothschild), *Catallagia borealis* Ewing, *C. onaga* Jordan.

The family Ischnopsyllidae includes the following eastern species: *Myodopsylla insignis* (Rothschild), *Eptescopsylla chapini* (Jordan), *Sternopsylla texana* (C. Fox).

THE FLUORESCENT BACTERIA IN DAIRY PRODUCTS[1]

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From the Department of Dairy Industry, Iowa State College

Received June 19, 1940

The fluorescent bacteria are important in the dairy industry because they grow rather rapidly at comparatively low temperatures and frequently cause deterioration of milk and its poducts during holding. Four hundred and ninety-six cultures of these organisms were isolated from dairy products, water and miscellaneous sources and their general characteristics studied. Selected cultures were also studied from the standpoint of their resistance to heat, chlorine and sodium chloride and their action on butter.

The attempts to isolate fluorescent bacteria from dairy products were much more successful when enrichment procedures, which involved holding the samples at 3° to 6°C. for several days before plating, were followed instead of plating the fresh products. The organisms were isolated from 207 (61.9 per cent) of 334 milk samples, 87 (58.4 per cent) of 149 sweet cream samples, 5 (4.8 per cent) of 104 sour cream samples, 7 (18.4 per cent) of 38 ice cream samples, 59 (31.9 per cent) of 185 butter samples, 56 (91.8 per cent) of 61 water samples and 15 (94.0 per cent) of 16 samples of miscellaneous materials.

The cultures isolated were all gram-negative, non-spore-forming rods with polar flagella.

The fluorescigenic ability of the cultures studied was affected by a number of factors, the most important being the composition and pH of the medium, the oxygen supply and the incubation temperature.

Absorption spectra studies indicated that the fluorescent pigment produced by these organisms is not fluorescin, fluorescein or riboflavin, and that fluorescence in alkaline culture media is not due to the formation of diacetyl.

All the cultures of fluorescent bacteria grew at 3° to 7°C. and at 32°C., but many cultures failed to grow at 37°C., although a few grew at 45°C.

The types of changes produced in litmus milk by the various fluorescent bacteria were (a) rapid proteolysis accompanied by a putrid odor, (b) rapid proteolysis with development of an amber colored solution and an indol-like odor, (c) alkaline reaction without proteolysis, (d) slight alkaline reaction followed by slow proteolysis, (e) slight alkaline reaction followed by an acid reaction but usually no coagulation and no reduction except at the bottom of the tube, and (f) formation of an acid ring with acid coagulation from the top down and reduction except at the surface; slight proteolysis and a May apple odor sometimes noted.

The cultures of fluorescent bacteria tested were still alive after 6 months in skimmilk at room temperature and after 6 months in unsalted butter at 1° to 3°C.

Milk cultures of fluorescent bacteria were regularly destroyed at a temperature of 57.2°C. for 30 minutes. The organisms, likewise, were not resistant to chlorine; in general they were destroyed within 5 minutes in water containing 10 parts per million available chlorine and within 2.5 minutes in water containing 15 parts.

Six per cent sodium chloride in beef extract-peptone broth inhibited the growth of some cultures of fluorescent bacteria but not of others; only a few cultures grew in broth containing 8 per cent sodium chloride.

All the cultures of fluorescent bacteria grew in beef extract-peptone broth over a pH range from 5.5 to 10.0 (the highest tried), while many cultures grew at a pH of 4.5 and four grew at a pH of 4.0. When the pH of the original broth was high the organisms tended to decrease it and when the pH was low they tended to increase it. The ten cultures tried, likewise, grew fairly rapidly in sterilized skimmilk adjusted to a pH of 5.0 and six of the cultures developed a bitter flavor in the milk after 96 hours at 21°C.

In skimmilk, the most proteolytic culture studied produced considerable increases in total nitrogen and amino nitrogen after 2 days at 21°C. and a considerable increase in ammonia nitrogen after 14 days, the first time that the ammonia content was determined. This culture also produced significant increases in total nitrogen and amino nitrogen within 14 days at 5°C.

In 7 days at 21°C., 47 of 52 cultures produced some type of flavor defect in unsalted butter churned (without butter culture) from sterile cream to which the organisms were added; at 1° to 3° C., 21 cultures produced flavor defects within 28 days. When butter culture was added to the cream before churning, flavor defects were produced in the butter held at 21°C. by 36 cultures, while when 2 per cent salt was added to butter made without butter culture, 25 cultures produced flavor defects. The off-flavors that developed in the unsalted butter made without the use of butter culture were unclean, cheesy, putrid, bitter, rancid and fruity; a combination of two or more of these flavors occurred in some butter samples. Two cultures produced color defects in the unsalted butter made without butter culture within 5 to 7 days at 21°C., one causing a bluish-black and one a salmon-pink color.

Fluorescent bacteria were isolated from 39 (34.5 per cent) of 113 samples of fresh sweet cream butter obtained from several different plants. Nineteen of the samples that contained fluorescent organisms developed flavor defects during 6 to 8 days at 21°C.; with 12 samples the off-flavors were pronounced and were evident after 2 to 4 days. Rancidity was the most important defect that developed. Both rancid and cheesy flavors occurred in some samples but a pronounced cheesy flavor unaccompanied by rancidity developed in only one sample.

Five out of six samples of cottage cheese, obtained from different

plants and held in water at 3° to 6°C., contained fluorescent bacteria, but the organisms grew sufficiently in only two samples to be a possible factor in the deterioration of the cheese during 6 to 10 days.

Two hundred and sixty-two of the 496 cultures of fluorescent bacteria isolated were identified as belonging to 10 different species in the genus Pseudomonas but the other 234 cultures could not be identified on the basis of the species described by Bergey, Breed, Murray and Hitchens[2]. Two hundred and thirty-one of the unidentified cultures resembled *Ps. fluorescens* except in their nitrate reducing and hemolytic abilities; the other three cultures were also proteolytic but produced a water soluble, brown pigment. The unidentified cultures were divided into Group I, Group II, Group III, and Group IV, but were not given species names.

[2] Bergey, D. H., Robert S. Breed, E. G. D. Murray, and A. Parker Hitchens. 1939. Bergey's Manual of Determinative Bacteriology, 5th Ed. The Williams and Wilkins Co., Baltimore.

THE PHENOMENON OF HOMOLOGY WITH LONG-CHAINED ALIPHATIC COMPOUNDS[1]

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The development of the concept of homology is reviewed and its application to long-chained aliphatic compounds is discussed. The physiological effects of a number of long-chained compounds are reviewed.

The investigations have provided certain new data relative to the limits of application of the phenomenon of homology, and concurrently there have been prepared compounds which might serve as derivatives for parent long-chained compounds, or which might exhibit some other useful property.

The following amides and sulfonamides were prepared from long-chained amines and the appropriate acyl chlorides: *N-n-octadecylbenzamide*, m.p. 85-87°; *N-n-octadecyl-p-toulenesulfonamide*, m.p. 89-91°; *N-(p-acetaminobenzenesulfonyl)-n-octadecylamine*, m.p. 130-131°; *N-n-octadecylstearamide*, m.p. 96-97°; and *N-(p-toluenesulfonyl)-di-n-octadecylamine*, m.p. 59-61°. Mild hydrolysis of N-(p-acetaminobenzenesulfonyl)-n-octadecylamine gave *N-(p-aminobenzenesulfonyl)-n-octadecylamine*, m.p. 118.5-120.5°. With phenyl isocyanate n-octadecylamine gave *N-phenyl-N'-n-octadecylthiourea*, m.p. 99-100°, and with phenyl isothiocyanate, *N-phenyl-N'-n-octadecylthiourea*, m.p. 86.5-88°. Attempts to prepare N,N-di-n-octadecylstearamide, N-phenyl-N'-di-n-octadecylurea, and N-(p-acetaminobenzenesulfonyl)-di-n-octadecylamine were unsuccessful. An attempt to prepare tetra-n-octadecylammonium iodide by heating tri-n-octadecylamine with n-octadecyl iodide gave *tri-n-octadecylamine hydroidide*, m.p. 79.5-81°. Pentaerthrityl bromide and n-octadecylamine yielded an unidentified compound (m.p. 98-100.5°) rather than the desired tetrakis(n-octadecylaminomethyl)methane.

Esters used in the synthesis of long-chained barbituric acids were prepared from diethyl ethylmalonate and long-chained alkyl bromides. Those prepared were diethyl ethyl-n-dodecylmalonate, diethyl ethyl-n-tetradecylmalonate, *diethyl ethyl-n-hexadecylmalonate*, b.p. 198-204°/2 mm., d_{20}^{20} 0.9078, N_D^{20} 1.4478, and *diethyl ethyl-n-octadecylmalonate*, b.p. 206-210°/2 mm., m.p. 38.5-40°. Condensation of urea with the esters gave *5-ethyl-5-n-dodecyl-barbituric acid*, m.p. 106-107.5°, *5-ethyl-5-n-tetradecyl-barbituric acid*, m.p. 104.5-106.5°; *5-ethyl-5-n-hexadecyl-barbituric acid*, m.p. 103-104°; and *5-ethyl-5-n-octadecyl-barbituric acid*, m.p. 108-109.5°. These compounds exhibit considerable depression in mixed melting points with successive homologs; therefore, their use as derivatives is proposed.

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Homologs of Phenacetin were prepared from long-chained-alkyl p-nitrophenyl ethers. The reaction of sodium p-nitrophenate with the alkyl bromides gave the intermediate ethers: *n-dodecyl p-nitrophenyl ether*, m.p. 48-49.5°; *p-nitrophenyl n-tetradecyl ether*, m.p. 58.5-61°; *n-hexadecyl p-nitrophenyl ether*, m.p. 63-65°; and *p-nitrophenyl n-octadecyl ether*, m.p. 68-71°. Reduction of the ethers followed by acetylation gave *p-acetaminophenyl n-dodecyl ether*, m.p. 92-93°; *p-acetaminophenyl n-tetradecyl ether*, m.p. 95.5-97°; *p-acetaminophenyl n-hexadecyl ether*, m.p. 96-98°; and *p-acetaminophenyl n-octadecyl ether*, m.p. 100.5-102°. These compounds are suggested as derivatives since there is a marked depression in mixed melting points of successive homologs.

Homologs of Dulcin were prepared by the reaction of potassium cyanate with p-alkoxyanilines. The two homologs prepared were [*p-(do-decyloxy)phenyl*] *urea*, m.p. 131-133°, and [*p-(tetradecyloxy)phenyl*] *urea*, m.p. 130-131.5°.

Attempts to prepare dihydrazides of long-chained malonic acids by reaction of mono-long-chain-substituted malonic esters with 100 per cent hydrazine hydrate were unsuccessful. Intermediate esters prepared from alkyl bromides and diethyl malonate were diethyl *n*-dodecylmalonate, diethyl *n*-tetradecylmalonate, and diethyl *n*-hexadecylmalonate. The physical constants (previously unreported) for the tetradecyl member were: b.p. 168-175°/2 mm., d_{20}^{20} 0.9189, N_D^{20} 1.4423.

The p-alkoxyanilines were diazotized in an aqueous medium and coupled with β-naphthol, giving *1-[p-(dodecyloxy)phenylazo]-2-naph-thol*, m.p. 80-82°; *1-[p-(tetradecyloxy)phenylazo]-2-naphthol*, m.p. 81-5-83°; and *1-[p-(hexadecyloxy)phenylazo]-2-naphthol*, m.p. 82-84°. Diazo-tization of N-(p-aminobenzenesulfonyl)-n-octadecylamine and the -*n*-dodecyl homolog in aqueous medium, followed by coupling with β-naph-thol gave *1-[p-(N-n-octadecylsulfonamido)phenylazo]-2-naphthol*, m.p. 158-159.5°; and *1-[p-(N-n-dodecylsulfonamido)-phenylazo]-2-naphthol*, m.p. 163-164°.

n-Dodecyl bromide and diphenylamine gave *N-n-dodecyldiphenyla-mine*, b.p. 198-202°/2 mm., N_D^{20} 1.5432. *n*-Tetradecyl bromide and thio-phenol gave *phenyl n-tetradecyl sulfide*, m.p. 39.5-41.5°.

Attempts to sulfonate tri-*n*-octadecylamine in acetic anhydride with concentrated sulfuric acid and with fuming sulfuric acid were unsuccess-ful. No sulfonation product was obtained with 20 per cent oleum alone.

Attempts to metalate N-*n*-dodecyldiphenylamine by refluxing with *n*-butyllithium in ether for 29, 51, and 72 hours gave no metalation prod-uct, and 93-96 per cent of the amine was recovered. Phenyl *n*-tetradecyl sulfide refluxed with *n*-butyllithium in ether for 52 hours gave only traces of benzoic acid upon carbonation; 64 per cent of the sulfide was recovered. With *n*-butyllithium in petroleum ether (b.p. 28-38°) phenyl *n*-tetradecyl sulfide gave no acidic product upon carbonation after 48 hours of refluxing, and 92.1 per cent of the sulfide was recovered. Hexa-decylbenzene (from Friedel-Crafts alkylation) with *n*-butylsodium in petroleum ether (b.p. 85-100°) at room temperature for 72 hours yielded

a small amount of acidic material upon carbonation; this has not been identified. N-ethyldiphenylamine with *n*-butyllithium in refluxing ether for 30 hours gave, subsequent to carbonation, an unidentified acidic product.

It is concluded upon the basis of the experimental results that the phenomenon of homology can be extended to the majority of the long-chained compounds included in these investigations.

COMPARATIVE STUDIES ON THE OXIDATION OF POYLHYDRIC ALCOHOLS BY BIOLOGICAL AND NON-BIOLOGICAL MEANS[1]

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The transformation of the simple sugars into ethyl alcohol by yeast and into butyl alcohol and acetone by bacteria has been known for a number of years, but only recently has the investigation of the milder oxidizing action of the Acetobacter or sorbose bacteria been intensively studied. The bacteria were classified by Kluyver and Donker (1924) as "aerobic organisms with strong oxidative (dehydrogenating) powers, characterized by the direct dehydrogenation of glucose to gluconic acid in the presence of powerful hydrogen acceptors such as oxygen and methylene blue."

Due largely to Bertrand's (1898) classical research with the sorbose bacteria their dehydrogenation action on polyhydric alcohols has been rather thoroughly investigated. Although more recent research has rather confused the problem of configuration in regard to dissimilation by Acetobacter, Bertrand's ideas on this subject are still generally accepted. He tested a number of polyalcohols in 2 per cent solutions containing 5 per cent yeast water and concluded that when compounds made up of primary and secondary alcohol groups, as are the polyhydric alcohols, are oxidized by the sorbose bacteria, only the secondary alcohol grouping is attacked and converted into the ketone group in the beta position, and the dehydrogenation depends on the configuration of the molecule. The favorable configuration is that in which the beta and gamma hydroxyls are in the *cis*-position. To support these conclusions Bertrand (1898) reported that glycerol, erythritol, *l*-arbitol, *d*-sorbitol, mannitol, persitol, and volimitol were dehydrogenated to beta-ketonic compounls, while *l*-xylitol and dulcitol were not attacked. The mechanism of the dehydrogenation is explained by assuming that the sorbose bacteria activate the beta hydrogen atoms in such a way that they are easily stripped off by oxygen or other hydrogen acceptors.

The action of *Acetobacter suboxydans* which was first reported by Kluyver and de Leeuw (1924) differs from that of the other acetc acid bacteria as regards intensity of oxidation. Their action is so mild that glucose is dehydrogenated to gluconic acid or 5-keto-gluconic acid and no further even when the culture is artificially aerated.

Fulmer, Dunning, Guymon, and Underkofler (1936) have studied the effect of concentration of sorbitol upon the production of sorbose by the action of *Acetobacter suboxydans*. They found that at an concentration of 35 per cent sorbitol, or below, approximately 80 per cent can

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be recovered as sorbose. The use of high concentrations of sorbitol affords an easy method for the production of sorbose in the laboratory and illustrates the mild action of these organisms.

This peculiar action of *Acetobacter suboxydans* toward primary and secondary alcohol groups on the same molecule leads one to believe that there must be some fundamental difference between these alcohol groups as regards their behavior in the presence of oxidizing agents.

It has been suggested that the preferential oxidizing action which the *Acetobacter suboxydans* shows toward secondary alcohol groups is due to the fact that secondary alcohol groups are oxidized at a lower potential than the primary alcohol groups of the same molecule. Cook and Major (1935), using the method described by Isbell and Frush (1931), reported that 5-keto-gluconic acid as well as gluconic acid was formed when glucose was oxidized at an anode in the presence of sodium bromide. This finding together with the statement that "the sugar alcohols were found to be oxidized to ketones and then the oxidation characteristic of ketones took place", made by Everett and Sheppard (1936) with reference to bromine, seems to substantiate the suggestion that the oxidizing action of the *Acetobacter suboxydans* is a matter of potential. In order to obtain data which would give some insight into the possible mechanisms of these oxidations a series of electro-chemical oxidations were carried out by using hydroxyl-containing compounds as depolarizers.

It was decided that oxidation of the alcohols at low current density would be more applicable in this case because a current of low amperage is easier to produce and control, and too, the heating effects are not nearly so great. The direct current source used was a vacuum tube rectifier which could supply a maximum of 250 m.a. at a maximum voltage of 450 volts. The metal electrodes used were cut from thin sheet metal and the carbon electrodes were in the form of rods. Alundum diaphragms were used to minimize mixing of the solution in the anode compartment with that in the cathode compartment. In all cases the solutions were agitated by motor-driven propeller stirrers.

Preliminary experiments were made using a variety of electrolytes and electrodes in solutions where compounds with only one functional group acted as depolarizers. It was found that carbon electrodes in the presence of halide electrolytes oxidized the secondary alcohol group in isopropyl alcohol, whereas lead electrodes in sulfate electrolytes, both in acid and neutral solutions, oxidize both primary and secondary alcohol groups. The course of each oxidation was followed by making analyses for acid formation and ketone formation, the latter by the Goodwin (1920) modification of the Messinger method.

Using the results of these preliminary experiments as a guide, polyhydric alcohols were oxidized and the course of the reaction followed by analyzing for acidity and reducing power. At the end of the reaction the resulting compounds could not always be conveniently separated from the original alcohol, so various specific tests were used to indicate what type of compounds were present. They are Schiff's

test for aliphatic aldehydes, Molisch test for carbohydrates, Selivanoff's test for ketoses, Bial's test for pentoses, and the phenylhydrazine reaction.

Certain impurities present in the solution in small quantities have been found to play a very important part in determining the course which a chemical reaction takes. In ordinary chemical reactions they are known as catalysts, in biological processes they are usually called enzymes, but in electrochemical reactions they are known as carriers. In the case of electrochemical oxidations they aid in the reaction by assisting the transfer of oxygen from the electrode to the depolarizer. A number of compounds were tried but only a very few appreciably affected the speed of the reaction.

The results of all these experiments may be summed up by the following statements:

(1) Isopropyl alcohol is oxidized quite readily to acetone at the temperature of melting ice by alkaline iodine solution while ethyl alcohol is oxidized only slowly.

(2) A lead electrode in the presence of a sulfate electrolyte will oxidize both primary and secondary alcohol groups equally well. However, a carbon electrode in the presence of a halide electrolyte oxidizes a secondary alcohol group very readily and a primary alcohol group almost not at all.

(3) Polyhydric alcohol solutions oxidized with lead electrodes in the presence of sulfate electrolytes reacted positively to Schiff's aldehyde test and negatively to Seliwanoff's test for ketoses. These tests would indicate that the alcohol molecule has been badly degraded.

(4) Polyhydric alcohol solutions oxidized with carbon electrodes in the presence of sodium bromide reacted negatively to Schiff's aldehyde test and positively to Seliwanoff's test for ketoses, indicating the absence of degradation.

(5) Bial's test for pentoses showed the presence of a pentose in the solution whose oxidation was catalyzed by vanadium pentoxide. This test also substantiated the Seliwanoff test in every case.

(6) The color tests used indicate that on very mild oxidation, polyhydric alcohols tend to be oxidized to ketonic compounds. However, in the presence of more vigorous oxidizing conditions the polyhydric alcohol molecule seems to be attacked at several points.

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GROWTH CORRELATIONS IN MAIZE SEEDLINGS[1]

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Iodent, Hopi Indian and several strains of hybrid corn were used in studies of the distribution of maize seedling growth among the coleoptile, plumule, first internode of the epicotyl and the roots.

Irradiation of germinating seeds, just breaking the pericarp, with sunlight or with carbon arc light did not affect the subsequent growth of the seedlings. Illuminating the coleoptile tip resulted in a sharp shift from internode to leaf and nodal root growth. Shorter periods of high light intensity were as effective as longer periods of low intensity. Exposures of five minutes at 100 f.c. significantly affected the correlative development of the seedlings. Irradiation with a sunshine carbon arc was somewhat more effective than irradiation with a Mazda lamp.

First internode growth was effectively inhibited by three decapitations of the coleoptile tip at four-hour intervals, or by the complete removal of the coleoptile, from seedlings grown in the dark.

The data from these experiments show that there is a positive correlation between growth of the first internode and growth of the coleoptile, and a negative correlation between first internode growth and development of the plumule. Auxin appears to be the main factor influencing these correlations. Many data indicate that auxin in the coleoptile tip may be destroyed or inactivated by light. In the normal growth of maize seedlings, as soon the coleoptile reaches the surface of the soil, the auxin supply is reduced by the action of light and the elongation of the first internode stops. Plumule growth is immediately accelerated. Hetero-auxin paste applied to the coleoptile during or immediately after an exposure to light sufficient to cause this growth shift, in a large measure nullified the effect of light.

Correlations between roots and tops of seedlings were less striking. Root growth was moderately depressed during the period of rapid auxin activation by the coleoptile, but was not reduced by relatively high concentrations of indoleacetic acid in a lanolin paste applied to the decapitated first internode stumps. Decapitation of the first internode increased root growth of seedlings in either dark or light, but normal plants in light at 100 f.c. consistently made better growth than the dark controls. The difference is not considered to have been due to photosynthesis, since 100 f.c. is usually below the compensation point for maize.

Growth of roots at the coleoptile node was positively correlated with plumule growth. Any exposure to light of sufficient intensity to

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check first internode growth and accelerate plumule growth also stimulated growth of roots at the coleoptile node. Histological studies showed that nodal root primordia became active in dark grown plants after four or five days of growth without exposure to light. Internode growth stopped and plumule development became active at the same time. It appears that after about five days of growth in the dark at room temperature, either the auxin precursor in the endosperm became exhausted, or the aging coleoptile tip became inactive. resulting in a decreased auxin supply and a shift in growth from first internode to plumule without outside stimulation. Presumably added auxin would delay this shift. Once made this change in growth, regardless of the cause, was largely irreversible, apparently because of the differentiation of the cells of the intercalary meristem.

It was observed that plants grown in the dark and plants grown under various light treatments had essentially the same total dry weights. It appears that light did not influence translocation of food materials from the endosperm, but was rather a factor in determining the place where the food materials were used. Auxin paste applied to the tips of decapitated coleoptiles apparently increased translocation from the endosperm. Auxin treated plants were usually larger than dark controls but the proportional distribution of growth was essentially the same.

Analyses of etiolated and illuminated plants suggest that the diffuse meristem of the first internode cannot readily convert simple organic nitrogenous compounds into the complex protein molecules of protoplasm without the aid of a continuous supply of auxin. The massed apical meristem apparently does not need an external supply of auxin to insure the utilization of soluble organic nitrogen. The presence of added auxin, on the contrary, inhibited apical growth The abundant supply of auxin during early seedling development is considered to be responsible for rapid utilization of soluble organic nitrogen by the diffuse meristem of the first internode, with resulting growth of this region. At the same time differentiation of the cells of the diffuse intercalary meristem region was prevented or delayed, and the leaf and root meristems were inhibited.

INTERMEDIARY DISSIMILATION OF CARBYHYDRATES BY THE COLI-AEROGENES BACTERIA[1]

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Although the physiology of the coliform bacteria has been the subject of investigation for a long time, this work was undertaken not only to extend our knowledge of the metabolism of these organisms but also to clarify some of the existing contradictions regarding their carbohydrate metabolism. Investigations of this type are of vital interest to those concerned with the classification of bacteria, and are likewise of importance with respect to the possible practical significance of the use of bacteria for the preparation of useful chemicals. The influence of environmental factors on the dissimilation of glucose, glycerol, pyruvic acid and several phosphoric acid esters of carbohydrates was studied.

Pyruvic acid is attacked anaerobically by either resting or proliferating cells of *Aerobacter indologenes* to form products normally obtained from glucose. Under ordinary conditions (pH 6.0-6.3) resting cells form 2,3-butylene glycol, acetic and formic acids, carbon dioxide and hydrogen. Growing cells form less 2,3-butylene glycol, considerable ethyl alcohol and small amounts of lactic acid. When the medium is kept at pH 7.0 or above, resting cells form only formic and acetic acids. When the cells are grown on a glucose medium, which is kept alkaline and then centrifuged, and placed on pyruvic acid substrate, the tendency to form 2,3-butylene glycol is decreased. The development of the mechanism for forming acetylmethylcarbinol and 2,3-butylene glycol is retarded at high pH.

Under ordinary conditions of metabolism resting cells of *Escherichia coli* and *Citrobacter freundii* do not form 2,3-butylene glycol from pyruvate but instead lactic acid is formed as a reduction product.

Hexosediphosphate is readily attacked by *A. indologenes* to yield products normally obtained from glucose under similar conditions. Phosphoglyceric acid and α-glycerophosphoric acid are attacked in the presence of glucose but the latter acid is attacked with difficulty. In the presence of 0.02 M sodium fluoride, the dissimilation of glucose by *Aerobacter*, although inhibited, proceeds with the formation of the usual products.

If the fermentation of glucose by Aerobacter is carried out at pH 7.0 or above, the formation of acetylmethylcarbinol and 2,3-butylene glycol is practically stopped. Large yields of volatile acids result and the formation of carbon dioxide and hydrogen is greatly suppressed. Under acid conditions, pH 6.0-6.3, the volatile acid yield is low, and the

carbon dioxide and 2,3-butylene glycol yields are high. Under these conditions, added acetic acid is reduced. Under alkaline conditions added acetic acid is not attacked and small amounts of succinic and lactic acids are formed. Complete carbon recoveries were not obtained in the alkaline fermentation. The unrecovered carbon is non-volatile, neutral and insoluble in ether. Indications are that it is a polymer of some intermediate formed during the breakdown of sugar.

When acetic or propionic acid is added to a glucose fermenting culture of *A. indologenes* at pH 6.0-6.3, the acids are reduced resulting in decreased hydrogen yield and an increase in 2,3-butylene glycol. Propionic acid is reduced to propyl alcohol and there is no five or six carbon glycol formed. There is a decrease in ethyl alcohol equivalent to the propionic acid reduced and a simultaneous equivalent increase in 2,3-butylene glycol. This suggests that when acetic acid is reduced, it acts similarly to the propionic acid and does not itself actually enter into the formation of the 2,3-butylene glycol.

Formic acid is more vigorously attacked in an acid medium than in alkaline medium, however, attempts to cause a reduction of acetic acid by cell suspensions of *A. indologenes* with formic acid as a hydrogen donator were unsuccessful.

When acetaldehyde is added to a glucose fermenting culture of *A. indologenes* which is kept at pH 7.0 or above, the aldehyde is converted to equimolar quantities of ethyl alcohol and acetic acid. When it is added to a fermentation at a pH 6.0-6.3, increases are noted in acetylmethylcarbinol, acetic acid and ethyl alcohol and a decrease in hydrogen.

Under similar conditions, propionaldehyde and butyraldehyde are reduced to the corresponding alcohols with some of the butyraldehyde going to butyric acid. There are small increases in carbon dioxide, small amounts of succinic acid are formed and decreases in ethyl alcohol are noted. There is no increase in acetylmethylcarbinol.

Fumaric acid added to a glucose fermenting culture of Aerobacter is reduced to succinic acid but no increased formation of 2,3-butylene glycol occurs.

Aeration and pressure aeration at three atmospheres pressure caused increases in acetylmethylcarbinol but the yield of acetylmethylcarbinol plus 2,3-butylene glycol remains the same as that of an anaerobic dissimilation. The yield of ethyl alcohol is diminished with a corresponding increase in carbon dioxide and acetic acid. Added acetic acid is reduced in spite of the increased oxygen tension. Gaseous hydrogen apparently is oxidized by oxygen under pressure but not when aerated at atmospheric pressure.

Cell suspensions of Aerobacter form large yields of succinic acid from glucose in contrast to growing cells which form none under usual conditions in an inorganic salts-glucose medium.

Cells of Aerobacter which have been grown in an alkaline glucose medium, ferment glucose much more rapidly in an alkaline than in an

acid medium. They are very sensitive to acid, and form little 2,3-butylene glycol. Apparently under alkaline conditions, the mechanism for forming 2,3-butylene glycol and reducing acetic acid does not develop. Fermentation of glucose at pH 6.0-6.3 by cells of Aerobacter grown in an alkaline medium gives products resembling the usual fermentation of glucose by *E. coli*.

In studying the fermentation of glycerol by the coli-aerogenes intermediates yields of trimethyleneglycol, ranging from 30 to 60 per cent of the fermented glycerol, are obtained in a mineral salts-glycerol medium. In the presence of calcium sulphite considerable acrolein is formed in fermentations by *Citrobacter freundii*. Attempts to demonstrate the intermediary role of acrolein in the formation of trimethyleneglycol by adding it to glycerol fermenting cultures were unsuccessful. Neither fumaric acid nor sulphite prevents the formation of trimethyleneglycol. Other products of the fermentation are carbon dioxide, hydrogen and acetic, formic, lactic and succinic acids and ethyl alcohol.

Contrary to previous results, four strains of *A. aerogenes* ferment glycerol in a mineral salts-glycerol medium and convert approximately 45 per cent of the fermented glycerol to trimethyleneglycol. Traces of acetylmethylcarbinol and considerable 2,3-butylene glycol are formed but no succinic acid. Other products are carbon dioxide and hydrogen, acetic, formic and lactic acids. On the basis of these results trimethyleneglycol formation cannot be used as a character to separate Aerobacter from the intermediate coli-aerogenes bacteria.

DISTRIBUTION OF PSEUDOMONAS FRAGI[1]

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Pseudomonas fragi appears to be one of comparatively few species that cause practical difficulties in dairy products. Because it is lipolytic and psychrophilic, it is particularly important in butter and other dairy products stored at relatively low temperatures. This investigation was undertaken primarily to supply data concerning its distribution and habitat.

Samples examined included those from normal and defective dairy products, dairy plant equipment and water supplies and water, soil, bedding, feed, hay, milking utensils, miscellaneous barn equipment, barn floors, dusty ledges and cows. Following an enrichment process (inoculation of samples into litmus milk and 7 to 10 days incubation at 5° to 10°C.) platings were made on beef infusion or beef extract agar to which nile-blue sulfate (1-10,000) and fat emulsion had been added. Promising colonies which developed on the plates were picked into litmus milk and those which produced a typical May apple aroma were saved for further identification.

Most of the samples originated in Iowa but a considerable number were obtained in Kentucky and a few in other states. Accordingly the data are presented in three sections.

From 5 to 21 samples were obtained at intervals from the milk supplies from 14 farms on delivery at an Iowa milk plant. *Ps. fragi* was found one or more times in the supplies of 10 (71.4 per cent) of the 14 farms. The percentages of samples from these 10 farms that yielded *Ps. fragi* ranged from 7.7 per cent to 42.9 per cent and averaged 20.6 per cent. The organism was found in seven (7.7 per cent) of 91 samples obtained in October, in 16 (22.9 per cent) of 70 samples in November and in 6 (40.0 per cent) of 15 samples obtained from December to May. Of 176 milk samples examined, 29 or 16.5 per cent yielded *Ps. fragi*. A sample of raw cream separated from milk delivered by some of the above producers also yielded it.

Samples of defective dairy products examined in Iowa yielded the organism in 20 (62.5 per cent) of 32 instances. These samples included butter, butter serum, pasteurized cream and raw skim milk. It was found in 14 (70.0 per cent) of 20 samples criticised as rancid or having a May apple odor.

Ps. fragi was found in only 6 (5.8 per cent) of 104 samples from equipment in 39 Iowa creameries and 1 milk plant.

Of 30 Iowa dairy plant water supplies examined, 3 (10.0 per cent) yielded *Ps. fragi.*

On nine visits during winter and spring months to 6 of the 14 farms supplying milk to the above milk plant, 137 samples were obtained. These samples were from water (19), soil (14), feed (17), bedding (9), cows (15), milking utensils (14), miscellaneous barn equipment (29), barn floors and ledges (19) and manure (1). The percentage of samples of each type yielding *Ps. fragi* were: water 57.9, soil 57.1, feed 47.1, bedding 88.9, cows 33.3, milking utensils 14.3, miscellaneous barn equipment 55.2, barn floors and ledges 63.2 and manure 100.0. In all, 71 (51.8 per cent) of the 137 samples yielded the organism.

Seventeen samples of milk delivered to a Lexington, Kentucky, milk plant in June did not yield *Ps. fragi,* but it was isolated from 16 (40.0 per cent) of 40 samples delivered to the same plant in December.

It was found in each of two samples of skim milk which developed a May apple aroma on storage in a refrigerator at Lextington.

Samples from water, soil, feed, miscellaneous barn equipment and barn floors and ledges obtained during the summer season on ten Kentucky farms yielded *Ps. fragi* in only 2 (4.1 per cent) of 49 instances. Both were samples of feed; one of grain and the other of hay. However, 99 similar samples obtained on nine Kentucky farms during the winter season yielded it in 37 (37.4 per cent) of the instances. The percentage of samples of each type yielding *Ps. fragi* were: water 75.0, soil 78.6, feed 16.7, bedding 50.0, milking utensils 0.0, miscellaneous barn equipment 19.0, and barn floors, ledges, etc., 36.8. In all, it was isolated from 39 (26.4 per cent) of 148 samples obtained on Kentucky farms.

Samples of barnyard soil were obtained from State Agricultural Colleges in 33 states in the United States. *Ps. fragi* was found in 23 (69.7 per cent) of these samples. Samples from 18 (90.0 per cent) of 20 states in the eastern portion of the United States (Minnesota to Louisiana and eastward) yielded it, while it was found in samples from only 5 (38.5 per cent) of 13 states in the western portion of the country.

Procedures used in identification of the cultures isolated showed the cultures to be consistent in a majority of their characteristics. A few varied in their ability to coagulate litmus milk, produce acid from arabinose and liquefy gelatin.

Aroma production by *Ps. fragi* was speeded up in litmus milk and peptone broth and on beef infusion or beef extract agar when a small amount of ethyl alcohol was added to the medium. Other alcohols tried were not effective for this purpose.

Distillation of peptone cultures, followed by extraction of the distillate with ether, showed the aroma material produced by *Ps. fragi* to be ether soluble. In relatively high concentration, the odor of the material left after evaporation of the ether suggested the odor of plum blossoms, but in relatively low concentrations it resembled the May apple aroma which is so characteristic of *Ps. fragi.*

CAROTENOIDS IN CORN GLUTEN[1]

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The possibility of making the endosperm protein, or gluten, of corn available for more widespread industrial use is largely dependent upon finding economical methods for purifying the crude material. The present work is intended to furnish a background for possible solutions to the problem of removing the carotenoid coloring matter which accompanies the gluten—a problem which any purification process would have to solve.

In particular it was desired (a) to learn if the carotenoids were the only pigments present and (b) to find (using the chromatograph) if the carotenoids were altered as the gluten is processed by present commercial methods. Such methods include subjecting the gluten to heat, dilute acid, and air oxidation.

The literature (1, 2) indicates that only three carotenoids—zeaxanthin, cryptoxanthin, and beta-carotene—are present in corn. The absence of non-carotenoid pigments was indicated, since it could be shown experimentally that the pigment in corn was completely extracted wtih solvents and completely absorbed on the chromatograph. Further, the gluten obtained directly after softening the cor kernel was completely bleached with enzymes.

The study of the isomerized carotenoids was more complex than anticipated. Recent literature (6) shows that the carotenoids are more sensitive to change than hitherto supposed, even continued standing or short heating of a pigment solution causing the formation of new pigments. Although there have been no published reports as to the action of dilute acid upon zeaxanthin, the main corn pigment, experiments with lutein (3, 4) suggest that numerous isomerized pigments would be formed. In view of these facts it was imperative to use only those experimental methods which would leave no doubt that any pigment isolated was actually present in the corn gluten and did not arise during the isolation process.

EXPERIMENTAL METHODS

The customary methods for pigment analysis were studied with the following results:

1. The presence of acidic and saponifiable materials in the pigment solutions prevented adsorption on milder adsorbents. Adsorption on stronger adsorbents was impractical since closely related pigments were

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not separated from each other and the subsequent removal of the co-adsorbed oils was not possible without color loss.

2. Partition between 90 per cent methanol and Skelly A, followed by alkali washing, made it feasible to adsorb the zeaxanthin pigments on $CaCO_3$. Ordinary powdered C.P. $CaCO_3$ was a poor adsorbent. $CaCO_3$ made by precipitation from concentrated solutions of $CaCl_2$ and Na_2CO_3 and mixed with Hyflo Super Cel was more suitable.

3. The cryptoxanthin pigments could be purified satisfactorily only by saponification. The recovery of pigments from soap solutions was improved by precipitating the soaps with salt water and centrifuging the separated soaps with solvent in the presence of concentrated alkali. The pigment solutions thus purified could be adsorbed on deactivated alumina (active material moistened with water and dried at 105°C.).

Since the saponification process was rather lengthy, it was impossible to be sure that isomerization had not occurred. Fortunately, the major pigment, zeaxanthin, and its heat-labile isomers were in the methanol layer and this fraction could be worked up in as little as ten hours. Evaporation of solutions was avoided by working on a smaller scale (200-300 grams of gluten) and by using a combination of benzene and Skelly A in extraction processes.

The procedure used for chromatographic analysis was essentially that outlined by Zechmeister (5). Petroleum ether (b.p. 28°-30°C.) alone or mixed with benezene (with additions of small percentages of acetone for developing) was used as the solvent; acetone and alcohol were used as eluting agents; the solvent was forced through the column under nitrogen pressure. The elution liquors were evaporated below room temperature and further purification and crystallization processes were conducted entirely at or below room temperature.

EXPERIMENTAL RESULTS

Employing the above modifications, the pigments were isolated from (a) whole corn, (b) gluten obtained from corn after softening the kernel by heating at 50°C. for two days with dilute aqueous SO_2, (c) gluten after acid hydrolysis to remove contaminating starch, and (d) "xanthophyll oil", the residue in the commercial solvent extraction process for obtaining zein.

For comparison, zeaxanthin and cryptoxanthin were isolated from Chinese Lantern plants[2] and the purified zeaxanthin was isomerized by heat, acid, and air under controlled conditions. From these latter experiments, the following data were found:

1. Heating zeaxanthin in benzene results in the formation of neozeaxanthins A and B, as shown by Zechmeister. The B isomer, as isolated, differed somewhat in properties from those described by him. Melting point 108°-109° (92°C.); adsorption maxima, 480 and 453 mμ (489 and 457½).

2. Eighty-three per cent of the zeaxanthin heated with dilute acetic

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[2] *Physalis Alkekengi.*

acid was recovered unchanged. Zeozeaxanthin A was the main isomerized pigment formed.

3. Heating zeaxanthin with dilute HCl results in the formation of at least ten new pigments. Neozeaxanthins A and B are formed in largest amounts, while about one-half of the zeaxanthin was recovered unchanged. Of the other pigments (all are oils) some lie above zeaxanthin in the chromatogram and others (five bands) lie below it. One of these latter was found to resemble cryptoxanthin both in partition and in adsorption affinity. On deactivated alumina it could not be distinguished from cryptoxanthin. On $CaCO_3$ the two pigments formed one broad band in which the cryptoxanthin was in the top portion while that derived from the zeaxanthin was largely in the lower lighter-colored portion.

4. Bubbling air through a solution of zeaxanthin in benzene and alcohol for two days at room temperature left at least 80 per cent of the pigment unaltered. A small amount of neozeaxanthin A or B as well as two ill-defined partially oxidized pigment bands were evident in the chromatogram.

From the experiments on corn it was found that:

1. In addition to the three pigments known to be present in whole untreated corn, traces of three pigment bands lying above zeaxanthin were found. Two of these were probably neozeaxanthins A and B formed in part during the extraction process, or possibly present in the corn itself.

2. In gluten isolated after softening the kernel, there were present a large number of pigment bands, most of which were above zeaxanthin in the chromatogramm. None of these pigments was present in large amount; the greater part of the extracted pigment in the zeaxanthin fraction was unchanged zeaxanthin.

3. The pigments in gluten subjected to acid hydrolysis to remove the starch are similar to those formed in the control experiment with zeaxanthin and dilute HCl. It was estimated that well over half of the zeaxanthin was transformed into other derivatives.

4. In "xanthophyll oil" both neozeaxanthins A and B were isolated, the latter in crystalline form (m.p. 116°-117°C.). Beta-carotene was isolated in crystalline form from this material.

<div align="center">DISCUSSION</div>

In none of these experiments on corn were there any large amounts of pigment bands present which could be identified as partial oxidation products. All of the major isomerized bands appeared to arise either from heating or acid treatment, as could be shown by mixed micro-adsorption, melting point, similarity in position in the column, color of the band, and adsorption spectra, as compared with derivatives prepared under controlled conditions.

The resistance of the carotenoids to oxidation as indicated by the above results is confirmed by the persistence of the yellow color of the

gluten. Although the material is exposed to air throughout the entire commercial process, the final product is not noticeably less yellow than that which may be obtained at the earliest stages.

The fact that one of the acid isomerized pigments arising from zeaxanthin is practically identical with cryptoxanthin in both partition and in behavior in the column indicates that the usual methods of analysis for the latter jigment as a measure of the vitamin A potency of corn products would not give true values in cases where the corn has been in contact with acid.

From the experiments carried out, as well as from the literature pertaining to the chromatographic process, it is suggested that the adsorption is controlled by acid-base factors in non-aqueous solutions. The basic role of the hydrogen ion in the aqueous adsorption is of course well known.

By assuming that the hydrogen ion or its equivalent is also a controlling factor in the non-aqueous chromatograph, then the following phenomena ar explainable:

1. The solvents used are neutral—neither basic nor acidic.

2. The adsorbents used are alkaline or acid adsorbing.

3. Alcohol, or acetone and acetic acid, cause development and elution in proportion to their "acid strength".

4. Carotenoids are adsorbed in proportion to their acidity. Esterification reduces the acidity and consequently the adsorption affinity.

5. The co-adsorption of two different compounds occurs because both have the same "acidity".

6. The difficulty of extracting the carotenoids from soaps arises because they are chromatigraphically adsorbed on the colloidal soap molecules.

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RING CLOSURE OF OPTICALLY ACTIVE SUCCINIC ACID DERIVATIVES[1]

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The relationship between optical rotary power and the electrical character of the groups attached to the asymmetric carbon atom has been noted by several investigators. Hixon and Johns[2] have classified organic radicals on the basis of electron-sharing ability as determined by the ionization constants of amines and carboxylic acids. Burch[3] found that there is a qualitative relationship between the molecular rotations of a series of alpha substituted ethylamines, and the electron sharing ability of the organic radicals. Bulbrook[4] found a similar relationship for a series of alpha substituted pyrrolidines.

The rotary power of compounds depends, however, on more factors than simply the electronegativity of the groups. Using ionization constants of organic bases as a measure of the electronegativity of the radicals, one finds that a radical substituted in the alpha position in the pyrrolidine ring gives a compound with an ionization constant nearly equal to that obtained by substituting the same radical in the alpha position in ethylamine. The rotatory powers of these compounds are, however, very different. From this it seems likely that the closed ring in the pyrrolidines has a great effect upon their rotatory powers.

The investigation undertaken was the study of the effect of ring closure, and of electron-sharing ability of organic radicals on rotatory polarization. Optically active derivatives of mono-substituted succinic acids were chosen for the work, since the straight chain compounds can be converted to cyclic anhydrides and cyclic amides without direct alteration of the asymmetric carbon atom.

The optically active succinic acids prepared were the following: methylsuccinic acid, phenylsuccinic acid, p-anisylsuccinic acid, o-chlorophenylsuccinic acid, and cyclohexylsuccinic acid. The preparation of the following series of derivatives was attempted for each optically active acid: anhydride, amic acid, N-methylamic acid, anilic acid, imide, N-methylimide, and anil.

The results of the measurements of the optical rotatory powers of the acids in alcohol and in acetone and for some compounds in benzene, ethyl acetate and chloroform are summarized as follows:

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[1] Original thesis submitted August, 1939. Doctoral thesis number 542

[2] Hixon and Johns,, *J. Am. Chem. Soc.* 49:1786. (1927).

[3] Burch, Doctoral thesis, No. 319. Library, Iowa State College. (1935) [Unpublished].

[4] Bulbrook, Doctoral thesis, No. 350. Library, Iowa State College, (1935). [Unpublished].

in" his entire crop and made little effort to control grasshoppers had the crop completely destroyed, while a neighbor who seeded only summer-fallow and protected this crop by proper use of tillage and poisoned bait had only 7 per cent less and harvested a crop worth $6.19 per seeded acre. Other striking contrasts were obtained in many areas.

By grouping blocks of farms according to types and intensities of infestations and to agricultural practices it was shown that the importance of individual control factors varied according to the particular situation, but that in every area the returns per seeded acre were significantly correlated with at least one control factor. With severe stubble infestations, that is, *Melanoplus maxicanus mexicanus* (Sauss.), the returns were highly correlated with the proportion of crop sown on uninfested fallow. With sod infestations of *Camnula pellucida* Scudd., the use of tillage to prevent invasion of nymphs, and the timely spreading of poisoned bait were more important than the method of seeding. When nymphs were poisoned on eggbeds less bait was required than if they invaded the crop.

Fall and spring plowing were equally efficient in reducing egg infestations in stubble fields and were more effective than shallow fall tillage, which in turn was superior to shallow spring tillage and no preparatory tillage.

In areas with severe stubble infestations the gross returns per seeded acre from crops on fallow were two to three times greater than from any crops sown on infested stubble. Crops sown with only shallow spring tillage or with no preparatory tillage generally had the most grasshopper damage and produced the poorest crops; farmers almost invariably condemned "stubbling-in" under these conditions. Only in some areas of heavy soil or where the stubble infestations were light did this latter practice prove profitable.

Seeding before May 15 resulted in higher returns than seeding after this date.

The control of grasshoppers in Saskatchewan in 1934 was both practical and economically sound even under conditions of very severe infestations and of drouth, according to 93 per cent of the farmers in this study. Successful control was closely associated with good farming practices and in most instances required little or no added farm expense. By proper adapting and timing of the normal agricultural practices excellent results were obtained with the minimum of cost, time, effort and poisoned bait.

On the basis of the present analysis the following control program has proven most efficient in minimizing crop losses from severe stubble infestations of grasshoppers and in producing better net returns. Farm operations should be carefully planned so that limited resources may be used to the best advantage. (1) Land already prepared, summerfallow and fall worked land, should be seeded. (2) Sufficient fodder or fuel should be reserved to prepare the new summerfallow; this will permit effective control of all nymphs on such fields and will ensure uninfested

land with a moisture reserve for the following year. (3) Plowing, preferably five inches deep, and seeding should be done only if resources are available beyond that required above. "Stubbling-in" should be avoided. In fact, in years of grasshoppers and of drouth it has frequently proven most profitable to restrict the crop only to prepared summer-fallow. The proper working of fields to be fallowed is essential for the most efficient control. Black guard-strips should be placed around all such fields before any nymphs hatch. These fields should preferably be worked as soon as possible after hatching is complete, and trap-strips of volunteer growth left at intervals throughout the fields; the nymphs which concentrate on these trap-strips may be easily poisoned with very little bait. Poisoned bait should be spread wherever grasshoppers are abundant.

In every grasshopper infested area the control program should be adapted to the particular conditions and should be based upon the combination of (1) planning of farm operations for the best utilization of available resources, (2) protective tillage and (3) poisoned bait. A well organized extension campaign is necessary for an effective control campaign.

HISTORY AND DEVELOPMENT OF AN EXPERIMENTAL BOB-WHITE MANAGEMENT AREA IN SOUTHERN IOWA[1][2]

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The purpose of this investigation was to secure data on the management of the eastern bob-white (*Colinus virginianus virginianus* (Linnaeus)) on an experimental game management area located in Decatur and Wayne counties in Southern Iowa. These data included information on as many as possible of the agricultural, economic, and social factors related to the production and harvest of a shootable surplus of bob-white year after year. This investigation continued from July, 1936, to March, 1940.

The game management area was divided for experimental purposes into the north area containing 4,739 acres and the south area containing 2,974 acres. The north area was designated as the pay shooting area because the farmers were permitted to charge $1.00 a day per man for hunting privileges. The south area was designated as a free shooting area because the farmers were not permitted to charge for hunting but instead were paid 10 cents an acre. Both areas were to carry out identical recommended game management practices.

The inventories of agricultural crops and livestock, game management practiced by the farmers, and method of land operation were organized and tabulated in such a manner that comparisons could be made between the north and south areas and between tenant and owner operators.

The three-year average percentages for the agricultural crops grown were: corn (*Zea mays* var.), 14.36 per cent; oats (*Avena sativa* var.), 8.08; wheat (*Triticum aestivum* var.), 1.60; rye (*Secale cereale* var.), 0.87; and sorghum (*Sorghum vulgare* var.), millet (*Setaria italica* var.), and legumes 3.01. The average percentage of meadow for the three years was 15.03 per cent; of pasture, 55.8% and fallow 1.39. Comparison of the three-year average percentages of agricultural land usage showed little variation between the two areas or between owner and tenant operators.

The livestock raised on the area were: horses, cattle, hogs, and sheep. The three-year average of acres of pasture per animal unit for owner operators in the north area was 4.14 acres, for tenants, 7.12 acres; for owners in the south area 5.34 acres and for tenants, 6.06 acres; the

average number of acres of pasture per animal unit for all operators in the north area was 4.99 and in the south area, 5.88 acres.

Designated game management practices in 1936 were: planting sorghum seed, planting Korean lespedeza *(Lespedeza stipulacea)* seed, setting out trees, fencing an eroding ditch or a feeding station, and constructing a feeding station. The materials for these practices were furnished the farmers without cost. Part of the designated game management practices were performed by 100 per cent of both owner and tenant operators in the south area and by 75 per cent of the owner operators and 67 per cent of the tenant operators in the north area. The payment of 10 cents an acre to the operators in the south area did seem to influence slightly the performing of the designated practices for the year 1936. The cost for performing these game management practices including materials and the acreage fee was 13.34 cents an acre for the south area and 3.47 cents for the north area. On the whole there seemed to be little difference between the amount and effectiveness of game management practiced by owner and tenant operators. The interest of the farmers in bob-white management showed an increase since in 1939-1940, when no acreage fee had been paid for two years, 20 farmers, 10 owners and 10 tenants, fed quail during the winter and only two had fed them during the winter of 1936-1937.

Seed-eating birds were observed competing with bob-white for food only during the late summer, fall and early winter of 1936.

Cooper's hawk *(Accipiter cooperi)* and the great-horned owl *(Bubo virginianus virginianus)* were the only birds of prey present in sufficient numbers that might have been predators of quail.

The principal fur-bearers on the area were: the Virginia opossum *(Didelphis virginiana virginiana)*, eastern raccoon *(Procyon lotor lotor)*, Mississippi Valley mink *(Mustela vison letifera)*, prairie spotted skunk *(Spilogale interrupta)*, Illinois skunk *(Mephitis mesomelas avia)*, and Northern Plains red fox *(Vulpes regalis)*. Of the 58 fox stomachs examined only one contained any evidence of quail. A total income of $146.45 was received during the season of 1936-1937 for fur bearers' pelts taken on 11 farms containing 1,996 acres which was an average of 7.33 cents an acre. The three most frequently taken fur-bearers on these farms were the Illinois skunk, one to 24.65 acres, the opossum, one to 66.53 acres, and the prairie spotted skunk, one to 90.72 acres.

Norway rats *(Rattus norvegicus)* were so numerous in 1939 that they destroyed from one to three acres of corn in the fields for many farmers.

An average of one domestic cat *(Felis libyca domestica)* for each 41.2 acres was on the area in 1938. Apparently this population was too high for its continuance since within a year most of the cats died of a disease which the farmers said was similar to distemper.

The average percentage of owner operated land in the north area for four years was 54.63 per cent, of tenant operated land 41.02; owner operated land in the south area was 27.04 per cent and tenant operated

land was 70.69. The average percentage of unrented land for the same years was 4.35 per cent in the north area and 2.28 in the south area. Social and economic conditions of the people were important factors in bob-white management.

During the period of the investigation three winters and one summer of adverse conditions for bob-white survival and production were experienced. In contrast to these unfavorable seasons two consecutive summers and two winters of exceptionally favorable conditions for the bob-white occurred. These severe winters caused calculated losses of 87.8, 74.6, and 55.2 per cent of the quail.

The lowest calculated population density for the area was one bird to 85.7 acres and the highest was one bird to 3.33 acres which was built up during three consecutive rearing seasons. The highest calculated percentage rate of recovery was 456.7 per cent which occurred during the season when the breeding population was the lowest and the lowest percentage rate of recovery was 84.5 per cent when the breeding population was the highest.

Hunting quail was permitted in the season of 1936 and closed until the season of 1939. The farmers allowed very little hunting in 1936 and 211 birds were reported taken by hunters in 1939. As far as is known no hunter on either area was charged for the privilege of hunting.

The farmer-sportsman relationships did not progress to the stage of successful pay hunting which may be the satisfactory solution of hunting privilege on privately owned lands.

THE SCORPIONS OF ARIZONA[1]

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During the course of this work the writer endeavored to collect and classify representative specimens of the scorpions of Arizona. In order to facilitate the taxonomic work the external morphology was described. The morphological terms peculiar to scorpions and those taken over from entomology have been defined, and many of the important structures are presented in twenty figures.

In the collection are specimens from every county in Arizona except Apache. No specimens have been taken from that county yet. In the collection the following previously named species are identified: *Centruroides sculpturatus* Ewing, *Vejovis spinigerus* (Wood), *V. boreus* (Girard), *V. crassimanus* Pocock, *Hadrurus hirsutus* (Wood), and *Diplocentrus whitei* Gervais. From the remaining material the writer here describes the following new species:

Centruroides gertschi. Two parallel, irregularly shaped black stripes on dorsum. On the carapace these stripes represented by only two elongated spots on the posterior median keels. No pit in the center of the basal piece. A discontinuous stripe on the ventral surface of the cauda and fifth caudal segment not darker in color than the rest. Specimens were taken from Clifton, Mesa, Nogales, Thatcher, and Warren.

Vejovis aquilonalis. First segment of cauda has no distinct, granular inferior keels. Carapace shorter than fifth caudal segment and slightly shorter than movable finger of pedipalp. Dorsum of a uniform orange-brown color. The specimen, a male, was taken 37 miles south of the Grand Canyon on highway 64.

Vejovis wupatkiensis. First segment of cauda has weak inferior median keels, but inferior lateral keels distinct and granular. Anterio-median border of the carapace broadly, but not deeply, emarginate. Movable finger of pedipalp much longer than the fifth caudal segment. Entire body orange yellow to light brown and frequently variegated slightly with darker brown. The specimens were taken at the Wupatki National Monument.

Vejovis confusus. Body orange to pale yellow. In some cases the terga have fuscous bands. Hands smoothly keeled and fifth caudal segment as large as, sometimes larger than, those in front. The specimens were taken from Coolidge, Mesa, Superior, Tucson, and Wickenburg.

Vejovis jonesi. Preabdomen and legs yellowish-brown. Pedipalps, carapace and cauda a darker brown. Carapace shorter than fifth caudal segment and shorter than segment one plus two. Movable finger of the

pedipalp shorter than fifth caudal segment. Anterio-median border definitely emarginate. Seven well defined, subcircular, middle lamellae. The specimen, a female, was taken at the Wupatki National Monument.

Vejovis lapidicola. Foundation color yellow to orange-brown. Trunk somewhat variegated with dark brown. Anterio-median border quite deeply emarginate. Fifth caudal segment more than twice as long as wide. Intercarinal spaces of inferior keels on this segment smooth to finely granular. Carapace longer than fifth caudal segment and as long as segment one plus two. Movable finger of the pedipalp shorter than fifth caudal segment. Middle lamellae vary from five to seven, very definite and subcircular. The specimens were taken one mile east of Flagstaff.

Vejovis vorhiesi. Foundation color yellowish-brown variegated with dark brown and black. Trunk marbled above but not below. Each tergum coarsely granulated, especially the posterior half. Two more or less yellowish-brown, smooth to finely granular, V-shaped marks, with apex directed medially, found laterad on the second to sixth terga. Fifth caudal segment hardly twice as long as wide. Intercarinal spaces on this segment coarsely granular and variegated with black and brown. Carapace longer than fifth caudal segment. Sixt to eight subcircular middle lamellae in pectines. The specimens were taken in Huachuca Mts., Santa Catillina Mts., and Tucson.

Hadrurus spadix. Entire dorsum a rich reddish-brown. This species closely resembles that of *H. aztecus* Pocock but differs in the following characteristics: Interocular triangle of female very smooth with a few granules on periphery. Entire carapace as dark, if not darker than, trunk. Ocular tubercle of female somewhat granular posteriorly. Entire male tubercle granular mesially. Last abdominal sternum coarsely granular. Entire upper surface of brachium coarsely granular. Movable finger always longer than carapace. Specimens were taken at Kingman, Grand Canyon, and Wupatki National Monument.

Superstitionia donensis. Entire body shiny and smooth due to the remarkable lack of granules and keel formation. Foundation color from a cream to a tan. Three longitudinal brown stripes, two lateral and one median, traverse the dorsum. Pectines have three relatively large, angular middle lamellae and six broad-bladed teeth. Fulcra conspicuous but not as large as middle lamellae. Two lateral eyes on each side of carapce. Sternum broader than long and with a longitudinal median furrow which is T-like anteriorly. The specimens were taken in the Superstition Mts.

This last species, in order to place it in the family Chactidae in which it belongs, required the creation of a new subfamily, which was called Superstitioninae. This new group has the following differentiating characters: The pedipalp fingers have non-overlapping oblique rows of granules on their inner borders. The movable finger of the chelicera does not have any teeth on its interior surface. The median groove of the sternum is T-like anteriorly. The anterior surface of the cauda is smooth and has a pigmented indication of a single inferior median keel.

Vejovis subcristatus Pocock, *V. punctipalpus* Wood, *V. flavus* Banks, and *Centruroides suffusus* Pocock, reported by other writers as having been taken in Arizona, are not represented in the collection made by the writer.

Experimental studies were made on pathological effects of the venom of different species. The venom of the following produced only a local swelling on white rats with apparently no general effect: *Vejovis spinigerus V. wupatkiensis, V. confusus,* and *Superstitionia donensis.*

On the other hand, the venoms of *Centruroides sculpturatus* and *C. gertschi* were found to produce a general neuropathic reaction which was fatal to white rats. These two species were also found to be responsible for the human deaths due to scorpion sting in Arizona. A Mexican serum was found to be effective in saving the lives of persons stung by these species.

Many of the life habits of scorpions are described. Scorpions are shown to be negatively phototropic, and positively thigmotactic. Some species are negatively geotropic while others are positively so. The food of the scorpion is shown to depend upon the direness of the need. As a rule the scorpions live on soft bodied insects and spiders. They are shown not only to suck the body juices of their victims but also to eat the sclerotized parts as well. They are apparently able to digest the hard parts of the insect bodies. The mating habits and parental instincts are discussed, but copulation was not observed. The first two molts are given as occurring within an average period of thirty-five days, while a half-grown scorpion did not molt for over a year.

A CORRELATION BETWEEN ADSORPTION OF HYDROGEN AND ACTIVITY OF SOME CATALYSTS CONTAINING OXIDES OF COPPER[1]

BRADFORD ROY STANERSON

From the Department of Chemistry, Iowa State College

The phenomenon of the adsorption of gases on solids was recognized very early in the study of chemistry. This was also true of the general characteristics of catalysis, but it wasn't until 1906 that Bone and Wheeler (1) recognized that adsorption played an important part in catalysis involving gaseous reactants and solid catalysts. Since that time a great deal of work has been done in an effort to determine the true relationship between adsorption and catalysis. Recently Menzel (2) found some new hydrogenation catalysts containing oxides of copper and since the adsorption of hydrogen on catalysts of this type had never been determined, the investigation described in this thesis was started.

EXPERIMENTAL

The adsorption of hydrogen at 100°C. on the catalysts was determined in an apparatus designed for isothermal adsorption and for pressures varying from 0 to 60 cm. The free space in the catalyst bulb was determined by measuring the volume of helium necessary to fill the bulb.

The hydrogenation of furfural in the liquid phase was carried out using the catalysts mentioned above. The apparatus used was a rocking type, copper-lined autoclave approximately three feet in length and having an inside diameter of about three inches. The original pressure used was 600 lb. ± 20 lb. and the maximum temperature was 222°C.

The catalysts used were Cu_2O samples made by the reduction of $Cu(NO_3)_2$ by means of dextrose. These samples were promoted with oxides of Mg, Ca, Sr, Ba, and the double promoter $CaO-V_2O_4$. A copper-chromium oxide catalyst prepared according to the directions of Conner, Folkers and Adkins (3) also was used.

RESULTS

A. The amount of hydrogen adsorbed by a Cu_2O sample promoted with CaO was determined at temperatures varying from 0° to 174°C. and it was found that there was no measurable adsorption at 0°, 96 per cent adsorption at 57.3° and 1.07 cm. pressure, and no significant change at the higher temperatures. The rate of adsorption increased as the temperature was increased.

B. The reversibility of hydrogen adsorption at 100° on a promoted Cu_2O sample was determined and it was found that about one-

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fifth of the hydrogen could be removed by reducing the pressure from 52.27 cm. to 2.81 cm.

C. The effect of promoters on adsorption was determined and it was found that from 0- to 700-fold increases could be obtained. BaO was the best promoter and the others were effective in the following order: CaO and V_2O_4, CaO, SrO and MgO.

D. Adsorption isotherms were run on catalysts that were also used in the hydrogenation of furfural. The results of the experiments are summarized below. The adsorption is given as the number of milliliters of hydrogen adsorbed per 8.6 g. of Cu_2O at 11 cm. of pressure, and the reaction rate is given as pounds decrease of pressure per five-minute interval.

TABLE 1. *Adsorption of hydrogen and catalytic activity of some catalysts containing oxides of copper*

Catalyst	Cu-Cr oxide	Cu₂O BaO	Cu₂O CaO V₂O₄	Cu₂O CaO*	Cu₂O CaO*	Cu₂O SrO	Cu₂O*	Cu₂O*	Cu₂O MgO	Cu₂O** CaO
Temperature at start of hydrogenation	139	167	157	157	172	171	165	177	172	——
Moles of hydrogen used..	3.24	2.73	3.24	2.75	3.25	1.8	2.69	1.29	3.28	None
Reaction rate (average) ..	32.6	25	28.7	8.8	22.7	7.4	11.7	6	17.7	0
Reaction rate (maximum)	70	37	40	16	45	12	18	10	35	0
Temperature at maximum reaction rate	157	185	173	216	203	200	198	202	206	——
Adsorption	80	67	40	22	3	0.5	0.1	0.08	0	0

* The two samples were prepared differently.
** Commercially made Cu_2O used.

CONCLUSIONS

1. For those catalysts investigated which contained Cu_2O, the adsorption of hydrogen takes place with such a velocity that equilibrium is reached in two hours or less if the temperature is 57°C. or above. The higher the temperature the more rapidly the equilibrium is reached.

2. Only a small fraction of the hydrogen that is adsorbed at 100°C. by catalysts that contain Cu_2O is released as the pressure is diminished.

3. Some substances which promote the catalytic action of Cu_2O have a very great effect upon the amount of hydrogen adsorbed by a given amount of catalyst. Of those investigated BaO, CaO, and the double promoter, CaO and V_2O_4, have the greatest effect. The effect upon adsorptive capacity is many times greater than the effect upon catalytic activity.

4. The divalent oxides of Ba, Ca, Sr, and Mg have a wide range in

their effect upon Cu_2O as an adsorbent of hydrogen. BaO is the best and the others follow in the order listed above.

5. A commercial grade of Cu_2O shows no adsorption of hydrogen even when promoted with CaO. Different samples of Cu_2O prepared by slight modifications of the Fehling's method of preparation have slightly different adsorptive capacities. This indicates that the method of preparation of Cu_2O is important in determining its capacity to adsorb hydrogen.

6. For the catalysts investigated there is a fair qualitative correlation between the adsorptive capacity of the catalysts and their catalytic activity based on the following points: (a) the temperature at which hydrogenation starts, (b) the moles of hydrogen used during hydrogenation, (c) the average rate of hydrogenation, (d) the maximum rate of hydrogenation, and (e) the temperature at which the maximum rate of hydrogenation occurs.

<div align="center">LITERATURE CITED</div>

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EFFECT OF AVITAMOSIS E ON REPRODUCTION AND VITAMIN E STORAGE IN THE TISSUES AND MILK OF GOATS[1]

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The discovery of vitamin E and the elucidation of its cause and effect relationship to reproduction in small laboratory animals have led to subsequent investigations for determining its value as a prophylactic against certain types of sterility in domesticated animals and man. It is an established fact that vitamin E is necessary in the diet for normal reproduction in rats, mice and for the hatchability of eggs. Sterility evoked by experimentally induced avitaminosis E can be corrected in the female rat by the administration of vitamin E in the form of wheat germ oil, and also by administration of alpha-, beta-, or gamma-tocopherol or their allophanates. The assumption has been made that a deficiency of this vitamin does occur extensively in farm animals under usual farm conditions, but the clinical success reported with wheat germ oil (vitamin E) therapy in combating certain types of temporary sterility among farm animals has been empirical in nature. No valid experimental data exist to indicate to what extent vitamin E does actually affect fertility among our domesticated mammals.

Because of the economic importance of sterility in farm animals and since experimentally induced avitaminosis E is known to exert a deleterious effect on fertility in small laboratory animals, an accurate evaluation of this accessory food factor in remedying sterility in farm animals seemed apposite. Once infertility had been definitely produced it would be possible to proceed effectively to appraise the alleged therapeutic value of vitamin E, or certain commercial by-products known to be rich in this vitamin such as wheat germ and wheat germ oil.

Six years ago the Iowa Agricultural Experiment Station initiated a project to study the effect of an avitaminosis E regimen in larger animals. The objectives were: First, to determine whether animals thus restricted required vtiamin E in the ration for successful reproduction; second, to study the effect of avitamosis E on the reduction of the amount of this factor occurring naturally in milk and certain body tissues; and third, to ascertain whether continued avitaminosis E during a reasonable length of time would induce nutritional sterility and if so, to determine the rational therapeutic value of wheat germ oil. Experimental evidence which could be obtained for the elucidation of these problems would be of inestimable value to the livestock breeder.

Although the ultimate intention was to use dairy cattle for experimental animals, it was thought wise to use first a smaller ruminant be-

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[1] Original thesis submitted June, 1939. Doctoral thesis number 518.

cause of the extensive nature of this problem. For reasons of economy of animals, time, help and feed, milk goats rather than dairy cows were used in this study. Seven female goats of mixed origin and breeding were purchased in the spring of 1933. Four proved their fertility by dropping live and healthy kids prior to going on experiment. The remaining three were young virgin does. These goats and their progeny were continued on trial and under observation for their reproductive performance until November 5, 1937, when the work with the live goats was terminated. With the exception of two animals of proven fertility all of the males used were reared from does in the experiment.

The procedure employed in caring for all goats throughout the four and one-half years of the experiment was designed to eliminate any likelihood of the animals receiving vitamin E by contamination or in the ration. The goats were caged in pens which had wire bottoms and sides mounted on wooden farmes. They were restricted continually to a standard ration which had been treated with ethereal solution of ferric chloride in amounts equal to one per cent by weight of the ration. A suitable apparatus was used for the evaporation and recovery of the ether. Following recovery of the solvent the treated feed was stored at approximately 50°C. for two or more weeks to accelerate the inactivation of vtiamin E. As a further precaution the treated and aged feed was biologically checked with rats by means of resorption gestations before being fed to the goats. The ferric chloride treated, aged and tested feed supplemented with vitamin E-free accessory food factors constituted the goat experimental ration. All goats had access continually to iodized salt and fresh water.

The biological tests for vitamin E were carried out by restricting weanling, virgin vitamin E-depleted, and resorption gestation, groups of rats for extended periods of time to the materials to be tested. The occurrence of sterility as a result of the diet indicated the absence of vitamin E, the production of litters or prevention of sterility as evidence of its presence in the test material. The technique devised for the treatment of large quantities of natural foodstuffs with ferric chloride was effective in producing an avitaminosis E regimen. Administration of the treated experimental ration and untreated accessory food factors (vitamins A, D and B-complex) to virgin vitamin E-depleted rats as well as to rats which had undergone a resorption gestation invariably resulted in nutritional sterility following conception as manifested by resorption gestations.

The efficacy of the method of treatment was further demonstrated by the administration of an ether extract of the treated ration to vitamin E-depleted resorption gestation rats and by prolonged restriction of weanling male and female rats to the experimental ration. Both methods of feeding resulted in nutritional sterility in the two sexes. Microscopic examination of the testes of the males revealed a complete degeneration of the seminiferous tubules while the females went through resorption gestations typical of insufficiency of vitamin E.

The possibility that the ferric chloride may have caused resorption gestations in the rats and thus invalidated the tests for vitamin E was checked. Administration of the untreated ration or the ferric chloride treated ration supplemented with wheat germ oil to resorption gestation rats which had been reared and restricted to the ferric chloride treated ration cured the nutritional sterility in the females and resulted in normal litters at term. Thus it apparently was established that the experimental ration was adequate in all recognized food constituents except vitamin E.

The reproductive performance of the male and female goats was observed through the experiment. The fertility of the bucks allowed to reach maturity was measured by the number of matings which produced offspring, or their virility was estimated by microscopic determination of the percentage of motility of their sperms. Also histological studies of the reproductive organs of the male goats were made. The fertility and fecundity of the does was compared with that of does at other stations reared under usual conditions.

The inhibitory effects imposed by the ferric chloride treated (avitaminosis E) ration invariably resulted in sterility in male and female rats. This phenomenon was not observed in the goats. Unlike rats, the reproductive behavior of male and female goats was unaffected, although both species were restricted to the same experimental ration. No unusual difficulty was experienced in having the seven parental goats multiply to 48 during the four and one-half years of the experiment. Of the 48 goats, 35 were born to mothers while restricted to the experimental avitaminosis E ration. The 21 gestations out of a possible 22 occurring among 15 does in the course of four breeding seasons resulted in the birth of 31 live kids and four others which were dead at birth but without any detectable abnormalities. The male and female goats reared to sexual maturity developed or reproduced regularly except in the case of four goats. A possible explanation of the slightly subnormal development of the four goats could not logically be attributed to vitamin E-deficiency.

It was demonstrated that a prolonged avitaminosis E regimen was effective in decreasing the amount of vitamin E usually present in the milk and certain body tissues of the experimental goats. Milk, butterfat, muscle and adipose tissue, which were obtained from the first and second generation male and female goats reared and restricted to the ferric chloride treated experimental ration, when fed in large quantities to avitaminosis E sterile rats produced resorption gestations typical of an insufficiency of this factor. Conversely, these same materials produced normal litters when supplemented with wheat germ oil. Likewise, feeding vitamin E-depleted rats milk or muscle tissue obtained from goats reared under usual farm conditions always rescued the gestation, resulting in the delivery of normal litters at term. All the substances tested were handled in the same manner, stored under essentially the same conditions for approximately the same length of time. The rats received the materials as supplements to a basal vitamin E-free ration. That the basal

regimen employed in these tests was devoid of the anti-sterility factor was demonstrated by the fact that negative control groups of resorption gestation or weanling female rats remained sterile or depleted themselves of their vitamin E reserves when restricted to the basal ration.

The observance of decreased body reserves of viamin E in the experimental goats thus was analogous to the conditions which occurred in rats restricted to the identical avitaminosis E regimen. On the other hand, unlike rats, goats apparently are able to reproduce through several generaions although restricted continually to a vitamin E-deficient ration. The markedly decreased quantity of vitamin E in their milk and certain of their body tissues had no detectable deleterious effect on their fertility or fecundity.

The evidence presented demonstrates that rations for goats do not require supplementation with vitamin E or concentrates of this factor, such as wheat germ oil or wheat germ meal, to insure successful reproduction. Whether the vitamin E requirements of other farm animals are comparable to those of the rat or are negligible, as in the case of the goat, requires further study.

A PRELIMINARY LIST OF IOWA ANTS

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Of considerable value in entomological work is the determination of the distribution of the various species of insects. This work, while carried on extensively for many insects of economic importance, is often neglected in the case of families of less economic importance, such as the Formicidae. That the Formicidae would be neglected to a great extent were it not for the fascination which their instinctive and behavioristic patterns hold for many men, and which occasionally leads them over into taxonomic phases, is without doubt. The taxonomy of North American Formicidae has been very well worked out by Wheeler, Emery, Forel, Mayr, Smith and others. Some new forms are still being described but these are mostly of subspecific or varietal rank. A great deal of the distribution has also been worked out, some of it by means of State lists. No list of Iowa ants has ever been published. The writer has been collecting ants and working on their identification for the past two years, and it seemed advisable, therefore, to present a list of species from the state, as incomplete and as circumscribed in its scope as it may be.

This list contains no species not actually seen by the writer and only five forms (*Formica truncicola* subsp. *obscuriventris* var. *gymnomma, F. sanguinea* subsp. *rubicunda* var. *sublucida, F. (Proformica) neogagates* subsp. *lasioides, Camponotus caryae* subsp. *discolor,* and *C. caryae* var. *minutus*) have not been collected by the author. These were identified from old material reposing in the College collection. Such material, mounted singly without regard to the colonial and polymorphic character of ants, and much as if they were beetles or solitary Hymenoptera, is often very difficult to determine. Ants should always be collected by the colony if possible and an attempt should be made to secure a representation of all the worker forms of the colony and also any sexual or winged forms which may be present.

At the present writing extensive collecting trips to the various corners of the state have just been completed and as a result many more species have been found which will be identified and listed in a later publication. One of these, *Eciton (Acamatus) nigrescens,*[2] a "driver ant," has been inserted in the present paper because of its importance as a distributional record.

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[1] The writer desires to express his appreciation to Dr. H. H. Knight, Professor of Entomology, Iowa State College, for much valuable assistance and encouragement in this study.

[2] M. R. Smith has recently shown *Eciton (Acamatus) schmitti* to be synonymous with this species.

The bluffs along the Missouri river, where several colonies of *nigrescens* were found as well as another still unidentified species of Eciton, present an interesting ecological change from the rest of the state. These bluffs, composed mainly of clay (loess) have very steep slopes and are therefore very quickly drained and quite arid. This region thus simulates the conditions of the arid southwest and it is not surprising that southwestern ant forms have become part of the fauna. Several other species have been found in these bluffs which are rare or absent in other parts of the state.

The greater part of Iowa's ant fauna seems to be eastern or northeastern in character. Undoubtedly Iowa once supported a widely distributed and extensive prairie ant fauna also, which has now been displaced and destroyed in a great part by cultivation. Only one species can be classed as a part of the western or Rocky Mountain fauna. This is a "thatching ant," a member of the *rufa* group of the genus *Formica*. It can be found in Iowa only in the extreme northwest corner of the state along the Big Sioux river.

FAMILY FORMICIDAE

SUBFAMILY PONERINAE

Genus *Ponera* Latreille

1. *P. coarctata* Latreille subsp. *pennsylvanica* Buckley. ☿ ♀ ♂ —Ames, Clinton.

Common near Ames; nesting under stones in small colonies.

SUBFAMILY DORYLINAE

Genus *Eciton* Latreille

Subgenus *Acamatus* Emery

2. *E. (A.) nigrescens* Cresson. ☿ —Little Sioux, Sioux City.

This ant is common in Iowa only in the bluffs along the Missouri river. Since *nigrescens* appears to follow these bluffs closely I believe that it also exists in northern Nebraska and southern South Dakota on the bluffs of the same river. Thus *nigrescens* has a much greater range than heretofore suspected.

SUBFAMILY MYRMICINAE

Genus *Stenamma* Westwood

3. *S. brevicorne* Mayr subsp. *diecki* Emery var. *impressum* Emery. ☿ ♀ ♂ —Ames, Arnolds Park.

Rare; nesting in very small colonies under stones.

Genus *Leptothorax* Mayr

4. *L. curvispinosus* Mayr. ☿ ♀ —Ames.

Fairly common; the nests are small and obscure. Several colonies were found nesting in dried, hollow stalks.

5. *L. curvispinosus* subsp. *ambiguus* Wheeler. ☿ —Ames.

Much rarer than *curvispinosus* s. str.; known only from individual workers caught in sweeping.

6. *L. fortinodis* Mayr var. *melanoticus* Wheeler. ☿ —Clinton.

A few workers were found crawling on trees along a small stream.

Genus *Cremastogaster* Lund

7. *C. lineolata* Say. ☿ ♀ ♂ —Ames, Clinton, Van Buren County.

Fairly common; nesting under rocks and logs. Some of the colonies are very populous.

Genus *Solenopsis* Westwood

8. *S. molesta* Say. ☿ ♀ ♂ —Ames, Clinton.

Common; often nesting apparently far from other ants as well as in the more preferred location near them.

Genus *Monomorium* Mayr

9. *M. pharaonis* Linn. ☿ —Ames.

A few workers were taken in the Science building at Iowa State College where they are a pest on cabinet specimens.

Genus *Strumigenys* F. Smith

Subgenus *Cephaloxys* F. Smith

10. *S. (C.) clypeata* Roger. ☿ ♀ —Ames.

Apparently the rarest of ants near Ames. One winged female found crawling beside a pathway on the Campus and one worker found under a stone are all that I have been able to secure.

Genus *Pheidole* Westwood

11. *P. vinelandica* Forel. ☿ —Ames, Clinton.

Common; it prefers to nest in open woodland under stones. It is quite common in town under sidewalks.

12. *P. pilifera* Roger. ☿ ♀ —Ames.

Less common than the preceding species. It nests in small crater nests in the ground and can be found only rarely under stones.

Genus *Myrmica* Latreille

13. *M. scabrinodis* Nylander var. *schencki* Emery. ☿ ♀ —Ames, Clinton.

Common; nesting under stones and in small crater nests in open woodland.

Genus *Aphaenogaster* Mayr

14. *A. tennesseensis* Mayr. ☿ ♀ ♂ —Ames, Clinton.

Common; nesting in rotten tree trunks and logs. I have only once taken it in a double nest with *A. fulva* Roger subsp. *aquia* Buckley, its temporary host.

15. *A. fulva* Roger subsp. *aquia* Buckley. ☿ ♀ —Ames.

Common; nesting in the ground under rocks and logs unlike *A. tennesseensis.*

16. *A. fulva* subsp. *aquia* var. *picea* Emery. ☿ ♀ —Ames, Clinton.

A darker, smaller variety of the preceding species.

SUBFAMILY DOLICHODERINAE

Genus *Dorymyrmex* Mayr

17. *D. pyramicus* Roger var. *niger* Pergande. ☿ —Ames.

Common on open grassy hills where the grasses and other plants are much like those of the original virgin prairie but not found elsewhere.

Genus *Tapinoma* Förster

18. *T. sessile* Say. ☿ ♀ —Ames.

Common; nesting in any available cavity under bark, leaves, rocks, logs, etc.

SUBFAMILY FORMICINAE

Genus *Prenolepis* Mayr

19. *P. imparis* Say. ☿ ♀ ♂ —Ames, Clinton.

Common, but rarely seen except in early spring and late summer and fall. Its crater nests are most numerous in deep woods.

Subgenus *Nylanderia* Emery

20. *P. (N.) parvula* Mayr. ☿ ♀ ♂ —Ames, Clinton.

Rather rare, nesting under stones in dry, sunny situations.

Genus *Lasius* Fabricius

21. *L. niger* Linn. var. *neoniger* Emery. ☿ ♀ ♂ —Ames, Clinton, Thompson.

This variety of *L. niger* seems to be even more numerous than *americanus* around Ames. Almost all of my specimens have proved to be *neoniger.*

22. *L. niger* Linn. subsp. *alienus* Förster var. *americanus* Emery. ☿ ♀ —Ames.

While not as common as *neoniger* near Ames, *americanus* is probably very common over most of the state.

Subgenus *Formicina* Shuckard

23. *L. (F.) umbratus* Nylander subsp. *mixtus* Nylander var. *aphidicola* Walsh. ☿ ♀ ♂ —Ames, Clinton.

Fairly common; it nests under logs and stumps buried deep in moist soil.

24. *L. (F.) umbratus* subsp. *minutus* Emery. ☿ —Ames.

Rare; I have taken only one colony which was nesting under a deeply buried stone in damp soil.

Subgenus *Acanthomyops* Mayr

25. *L. (A.) interjectus* Mayr. ☿ ♀ ♂ —Ames.

The most common hypogaeic ant near Ames. One colony, nesting under the cement floor of a warm basement in Ames, carried on wedding flights during the month of January, 1939.

26. *L. (A.) latipes* Walsh. Beta-form ♀ —Ames, Clinton.

I have never taken the workers of this species and know it only from several of these peculiarly formed females found crawling about after their wedding flight.

27. *L. (A.) claviger* Roger. ☿ ♀ —Ames.

Fairly common, nesting as *interjectus* does under rocks and logs buried deep in damp soil.

Genus *Formica* Linn.

28. *F. fusca* Linn. var. *subsericea* Say. ☿ ♀ ♂ —Ames, Jewell, Clinton, Van Buren County.

Common; prefers to nest in lawns and open woodlands.

29. *F. truncicola* Nylander subsp. *obscuriventris* Mayr var. *gymnomma* Wheeler. ☿ —Ames, Red Oak.

Evidently very rare. I have been unable to take this ant myself and all my specimens are from old unidentified material in the College collection.

30. *F. sanguinea* Latreille subsp. *rubicunda* Emery. ☿ ♀ —Ames.

This subspecies of *F. sanguinea* is the more common one in woodlands. It is rare within city limits.

31. *F. sanguinea* subsp. *rubicunda* Emery var. *sublucida* Wheeler. ☿ — Ames.

Apparently very rare. I have only two old specimens from the College collection which I can assign to this variety.

32. *F. sanguinea* subsp. *subintegra* Emery. ☿ —Ames.

Fairly common for a slavemaker within the city of Ames. It is rare, however, in woodlands.

33.   *F. cinerea* Mayr var. *neocinerea* Wheeler. ☿ —Ames, Jewell.

This ant was found nesting commonly in the tops of boggy hummocks around the shores of Goose Lake and Little Wall Lake near Jewell and also rarely in lawns at Ames.

Subgenus *Proformica* Ruzsky

34.   *F. (P.) neogagates* Emery. ☿ —Ames.

Common in lawns at Ames; I have never taken it outside the city limits, however.

35.   *F. (P.) neogagates* subsp. *lasioides* Emery. ☿ —Ames.

Apparently very rare. I know this species only from old College material.

36.   *F. (P.) neogagates* subsp. *lasioides* var. *vetula* Wheeler. ☿ ♀ —Ames.

More common than the preceding form but still rare; it nests under stones in open woodlands.

Subgenus *Neoformica* Wheeler

37.   *F. (N.) pallide-fulva* Latreille subsp. *schaufussi* Mayr var. *incerta* Emery. ☿ —Ames, Clinton.

Rather rare, nesting under stones and in crater nests in open prairie.

38.   *F. (N.) pallide-fulva* subsp. *nitidiventris* Emery. ☿ —Ames.

The rarest of our three forms of *Neoformica*, near Ames at least.

39.   *F. (N.) pallide-fulva* subsp. *nitidiventris* var. *fuscata* Emery. ☿ ♀ —Ames.

Common; nests under stones and logs in open woodlands and prairies. It is also fairly common in lawns in Ames.

Genus *Polyergus* Latreille

40.   *P. rufescens* Latreille subsp. *lucidus* Mayr. ☿ ♀ —Ames.

Fairly common for a slavemaker in the lawns of Ames. I have not taken it outside city limits, however. Colonies of this species have been observed but not taken at Clinton and Davenport also.

Genus *Camponotus* Mayr

41.   *C. herculeanus* Linn. var. *pennsylvanicus* DeGeer. ☿ ♀ ♂ —Ames, Clinton, Van Buren County.

Common; it nests with equal facility in live trees as well as stumps, logs, and sometimes frame houses.

42.   *C. herculeanus* subsp. *ligniperdus* Latreille var. *noveboracensis* Fitch. ☿ ♀ —Ames, Clermont.

Rare near Ames, nesting in the same situations as *pennsylvanicus*.

43. *C. castaneus* Latreille subsp. *americanus* Mayr. ☿ ♀ ♂ —Ames, Clinton.

Fairly common; usually nests under stones or logs in the ground rather than in wood like *herculeanus*.

44. *C. caryae* Fitch. ☿ ♀ ♂ —Ames.
Rare; nests in dead branches.

45. *C. caryae* subsp. *discolor* Buckley. ☿ —Ames.
Rare; nests in the same situations as *caryae* s. str.

46. *C. caryae* subsp. *discolor* var. *clarithorax* Emery. ☿ —Ames.
Rare, a darker variety of the preceding subspecies.

47. *C. caryae* var. *minutus* Emery. ☿ —Ames.
Rare; this variety as well as *caryae* subsp. *discolor* was determined from old material in the College collection.

48. *C. caryae* subsp. *subbarbatus* Emery. ☿ ♀ —Ames.
Rare; I have taken only one colony of this species. They were nesting in rather damp ground in open woodland underneath an old board.

THE QUANTITATIVE SPECTROSCOPIC ANALYSIS OF SOILS

G.W. FOX AND R. A. GOODWIN[1]

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The spectrochemical method has been applied to a wide variety of problems and materials. However, only a few investigations have been made on soils or soil solutions. Flint (2) has reported on the application of spectrographic analysis to agricultural chemistry but does not give specific information regarding the analyses.

Lundegardh (7) has determined Ca, Mg, K, Na, and Fe in various soil extracts by means of their flame spectra. The flame technique was also used by Mitchell (9) for the determination of Ca, Mg, Na, Fe, Mn, K, Sr, and Li in soil solutions.

Milbourn (8) has analyzed soils as a dry powder. His method of analysis involved the use of a 7.5 ampere carbon arc, with arc gap of .75 mm., and synthetic samples which were prepared by adding a solution of re-crystallized borax to a mixture of two parts silica and one part alumina.

Oertel (10) investigated the possibilities of spectrographic analysis of soils using a condensed spark as the spectroscopic source. He concluded, using the internal standard method (3) and the ratio quantitative system (6), that the minor constituents of the soil could be determined independently of varying amounts of the major constituents.

Other works of interest to the soil spectroscopist are those of Rusoff, Rogers, and Gaddum (11) on the determination of copper in wire grasses, of Brunstetter, Myers, Wilkens, and Hein (1) who analyzed some 436 samples of grasses and legumes spectrographically, of Scribner (12) on the detection of rare earths in plants, and of Konishi and Tsuge (4) on the mineral contents of certain leguminous crops.

In this investigation an attempt has been made to analyze soils and soil solutions using a direct current carbon arc as the spectroscopic source by the use of the method of internal standards (3).

SPECTROSCOPIC EQUIPMENT AND PROCEDURE

The instrument used was a Bausch and Lomb medium quartz spectrograph in which the entire spectrum from 7000 Å to 2100 Å is photographed on a single plate 10 inches long.

The spectrographic carbons were manufactured by the Dow Chemical Co. and fulfilled the highest standards of purity. The rods were cut at right angles to their length, the ends made flat and smooth and the corners rounded off with a file. Spectroscopic examination of the electrodes after this treatment failed to show any contamination. Some electrodes had a

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small depression hollowed by a lathe tool into which dry samples could be packed.

The arc was excited by a 500 volt storage battery with sufficient adjustable ballast to vary the arc current from 2 to 10 amperes. An arc gap of 1 mm. and a current of 4.8 amperes was adopted as standard for the dry samples and a gap of .85 mm. and 2.5 amperes for the solutions.

The photographic densities of the spectral lines were measured by a modified Moll type microphotometer, only the optical system of which was used. Instead of recording the galvanometer deflections photographically they were read directly on a wall scale. Most of the lines examined were in regions where background was a minimum and no attempt was made to correct for it.

Wratten and Wainwright panchromatic plates were used throughout the investigation. These were developed in one part fresh Eastman D-72 stock solution and two parts of water at 18°C.

The test element lines were of such intensities that all the analyses could be made from a single exposure without a condensing lens except in the case of silicon. The intensities of most of the available lines were measured and working curves for each pair of lines of interest were prepared. By a working curve is meant a graph in which the log ratio of intensity of a test element line to the intensity of an internal standard line is plotted against log percentage composition of the test element. The percentage composition of a test element in the unknown is read directly from the working curve after the log ratio of two corresponding lines has been determined.

ANALYSES OF POWDERS

Several months were spent investigating the technique for analyzing the soil as a dry powder. In contradiction to the statements of Oertel (10), preliminary examination indicated that variation in the relative amounts of silica and alumina in the samples was markedly affecting the spectra of the minor constituents, therefore, it appeared advisable to determine the relative amounts of silica and alumina in a soil before attempting an analysis of the minor constituents. Considerable difficulty was experienced in preparing samples in which the silica was uniformly distributed but, after trying a number of schemes which failed, the following proved satisfactory. A sample of pure crystalline quartz was carefully ground and pulverized, and then shaken up in distilled water. After one hour, the liquid was decanted and, upon evaporation of the liquid to dryness, the residue furnished a supply of very finely divided silica which was used in the preparation of various synthetic soil mixtures.

Samples were prepared which contained from 0.1 to 2.0 per cent silica and appropriate amounts of other soil constituents. The matrix, which constituted more than 97 per cent of the total weight of each sample, was very pure $Sr(NO_3)_2$.

A known weight of $Sr(NO_3)_2$ was added to a sample of actual soil from which the organic matter had been removed so that the estimated

silica content would lie within the range covered by the synthetic mixtures. Sn and Zn were used as internal standards.

Repeated analyses were made on the same soil sample. For these, 15 mgm. samples were placed in a drilled depression in the positive electrode. The data showed that, when Sn was used as the internal standard, the silica and alumina determinations agreed within 15 to 20 per cent. In some cases, the agreement was within 10 per cent for four determinations. However, even with the utmost of care, divergent results occurred too frequently to say that the method could insure even 20 per cent accuracy for a single determination.

ANALYSIS OF SOLUTIONS

The soil solutions were prepared as follows: A known weight of dry soil, about 15 grams, was placed in 500 cc. of solvent-water, dilute HCL or NH_4OH. The mixture was kept at room temperature and stirred frequently for 24 hours. After being filtered, the solution was evaporated to a volume less than 100 cc. and 3.75 grams of very pure $NaNO_2$ dis-

TABLE 1. *Lines used and range of concentrations covered in the analysis of soil solutions*

| Element | Spectral lines | | Range of analysis |
	Element test A	Mo A	Percentages
Si	2881	3112	.00005-.008
Si	2881	3132	.0003 -.01
Si	2881	3170	.0003 -.01
Al	3092	3112	.0005 -.04
Al	3092	3170	.001 -.50
Al	3092	3132	.001 -.50
Al	3944	3903	.01 -.30
Al	3961	3903	.01 -.30
Fe	3020	3112	.0001 -.01
Fe	3020	3132	.001 -.10
Fe	3020	3170	.001 -.10
K	3217	3132	.001 -.10
K	3217	3170	.001 -.10
Ca	3933	3903	.0001 -.15
Mg	2795	3132	.00010-.10
Mg	2795	3170	.0001 -.10

solved in it. Finally, 5 cc. of ammonium molybdate solution, containing 0.2 per cent Mo, was added and the volume adjusted to 100 cc. by the addition of distilled water.

Several series of standard solutions were then made in which the percentage composition of the test elements to be determined was varied
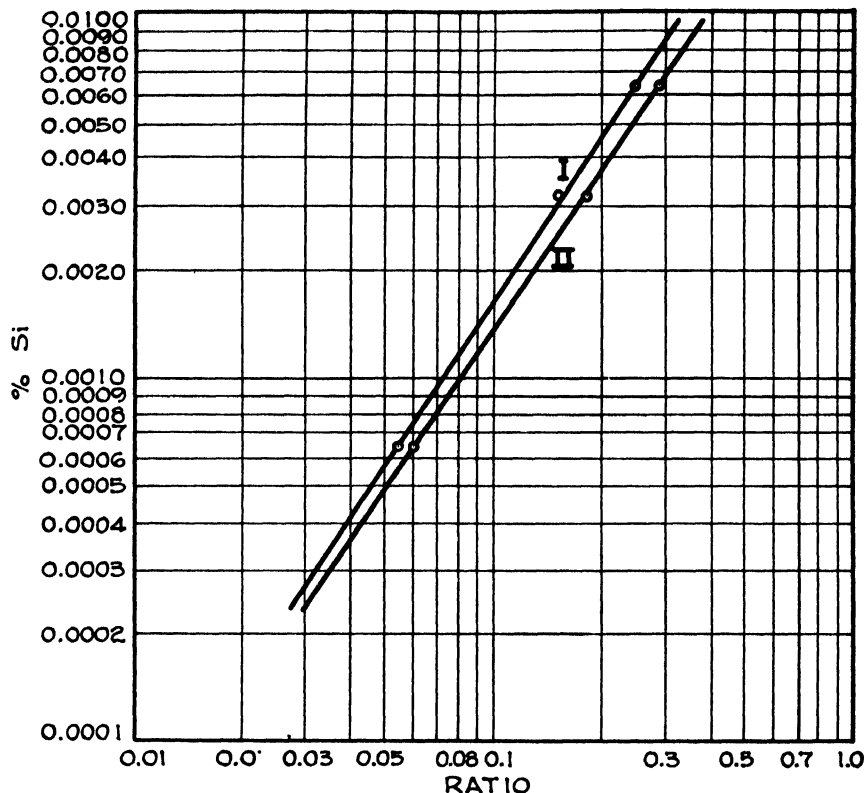
Fig. 1   WORKING CURVES FOR SI

$$I \sim \frac{Si\ 2881}{Mo\ 3132} \qquad\qquad II \sim \frac{Si\ 2881}{Mo\ 3170}$$

over a wide range while sufficient Na and Mo were added to make their concentration the same in these solutions as in the soil solutions described.

A drop of the solution to be analyzed was transferred to the end of a carbon test electrode by means of a glass rod and the electrode dried quickly in an oven after which the impregnated carbon was excited as the positive arc electrode.

The lines used and the range of concentration of the elements quantitatively determined are shown in table 1.

RESULTS AND DISCUSSION

Working curves were made for the quantitative determination of Si,
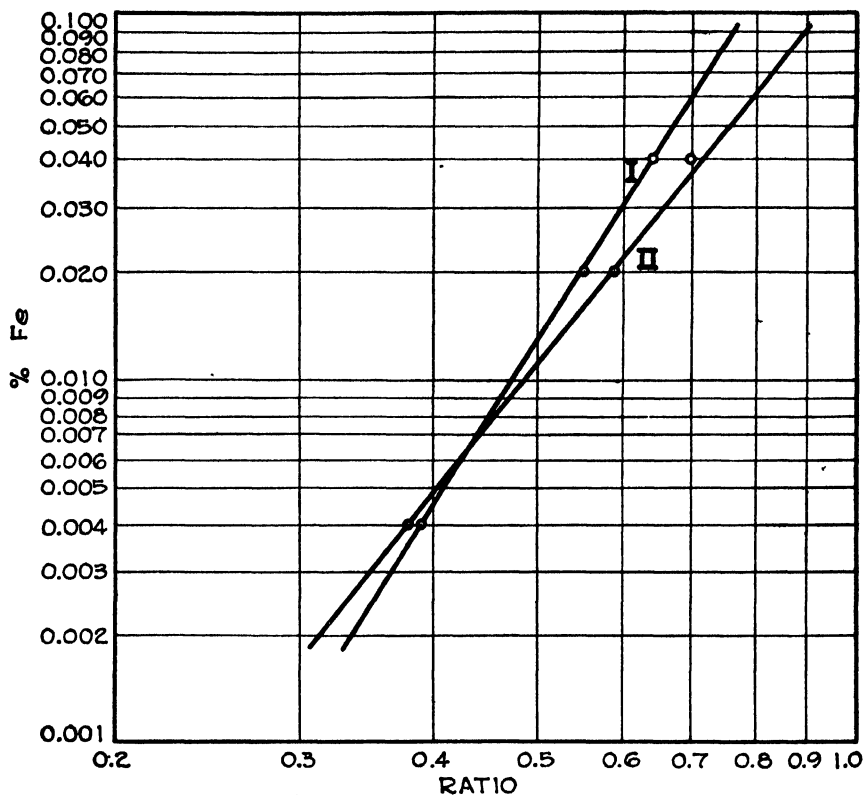
Fig. 2 WORKING CURVES FOR Fe

$$I \sim \frac{Fe\ 3020}{Mo\ 3132} \qquad\qquad II \sim \frac{Fe\ 3020}{Mo\ 3170}$$

Fe, Mg, K, and Ca and representative ones for Si and Fe are shown in figures 1 and 2.

The range of analyses as shown in table 1 does not in any sense indicate the upper or lower limit of the use of the spectroscopic method. They indicate only the range investigated by the writers.

Although the method of analysis here utilized has been successful in

the determination of certain elements in soil solutions, it has several objectionable features. In the case of Si, Al, Fe, Mg, K, and Ca the accuracy is between 10 and 15 per cent. Greater accuracy than this might be attained if the technique were applied to the routine analysis of soil solutions but the 10 to 15 per cent accuracy is a tolerable figure for a method using a direct current arc.

In its present state of development, the technique herein described is incomplete in several respects. A more thorough investigation of the effect of organic matter upon the analyses is needed. Also, a study should be made of the effects of the addition of a large excess of some element with a low ionization potential to the sample as recommended by Langstroth and McRae (5) to stabilize the arc. Such additions may have an undesirable effect upon the limits of detection of the various elements.

The analysis of soil solutions should be no more complicated than the analysis of any complex solution, though it is true that many spectral lines of the soil elements lie in a region not available in a method using a dc carbon arc. The cyanogen bands obliterate a large portion of the region between 3800 A and 4200 A. This difficulty could be minimized by using a different method of excitation such as the high voltage ac ε c which at present seems to be the favorite in industrial work. Further work on soil analysis is in progress with this type of source.

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EFFECTS OF DIETARY MODIFICATIONS OF HOST RATS ON THE TAPEWORM HYMENOLEPIS DIMINUTA[1]

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I. USE OF TRIBOLIUM CONFUSUM AS A VECTOR; LIMITATION OF THE AMOUNT OF FOOD

Among the results on the effects. of intestinal parasites of starvation of the host is that reported by Levine (35), who observed that the number of discharged segments of the poultry cestode *Davainea proglottina* was decreased for a period of a week following 24 hours of starvation of the hosts. On the other hand, Hegner and Eskbridge (26) kept six rats showing infections with intestinal trichomonads without food for eight days, and, at the end of this period, all the hosts were still infected and four of the infections were considered heavy.

In the investigation here reported, fifteen young rats of the Wistar A inbred strain[2] were placed in separate cages of hardware cloth and supplied freely with water and a ration made up as follows: beet sugar, 63 per cent; casein[3], 15 per cent; Celluflour, 3 per cent; complete salt mixture[4], 4 per cent; cod liver oil, 2 per cent; lard, 3 per cent; Fleischmann's yeast, 10 per cent.

After a preliminary examination of the pellets in which no tapeworm eggs were found, the animals were fed cysticercoids of *Hymenolepis diminuta* through the use of the flour beetle *Tribolium confusum* as a vector. Gravid proglottids from the posterior portion of rat tapeworms were mashed with a small amount of wheat middlings and furnished as food to confused flour beetles. After a period of three weeks to permit the development of cysticercoids in the intermediate hosts, the heads were removed from the beetles thus exposed to infection and both bodies and heads of about a dozen beetles added to the food of each rat. In order to insure prompt ingestion of the vectors, rats were deprived of food for a half day, then given only a small amount of food containing the dead beetles.

Fifteen days after the feeding of the intermediate hosts, enameled pans were placed under the cages. The one-half inch mesh of the hardware cloth used for the floors of the cages permitted the rat pellets to drop

through and be caught in the pans containing half an inch of water in which was dissolved a small amount of trisodium phosphate which softened the pellets somewhat and retarded bacterial growth. Every two days the pans were removed and replaced by clean ones, and the contents of each pan, containing the entire number of pellets for the preceding two days, were examined for tapeworm eggs. Three of the fifteen rats did not show eggs in the droppings by the thirtieth day and were considered to be uninfected and were not used further. The other twelve began to eliminate eggs within 17 to 24 days after ingestion of the cyticercoids.

The collected pellets were broken up and the material from each pan was thoroughly mixed with an electric beater and diluted with water to one liter. This suspension was then poured from one container to another to insure, insofar as possible, a homogeneous mixture. Immediately a small sample was poured off and thoroughly mixed; from it a small amount of fluid was drawn into a pipette fitted with a rubber bulb, and dropped into a mold counting chamber of known volume. The entire area of the counting chamber was examined and the number of eggs present determined, from which the number of eggs in the entire liter of material, and the number eliminated by each rat each day, could be calculated.

Collections of material and counts of tapeworm eggs were made every other day for each rat during the course of the experiment.

For 29 days the young rats had free access to the diet described above while determinations of the numbers of eggs being passed were made every two days. For the last week of this period the weight of the food eaten each day by each rat was determined. One-half of this average daily intake was given to each of six experimental rats (designated in this study as D2, D3, D4, D8, D10, and D12) each day for the next two weeks, while the other six control rats (D5, D6, D7, D13, D14, and D15) were given the same ration but in any desired amount. The weight of the feed eaten by these control animals was determined each day. From the sixth to the ninth week, each experimental animal was given only one-third the average amount of the feed eaten by the control animals during the preceding week. At the ninth week the groups were reversed in treatment; rats D2, D3, D4, D8, D10, and D12 being given food ad libitum and the other six allowed only one-third the average amount eaten by the control animals. This procedure was continued for three weeks when the experiment was terminated.

RESULTS

When the diet was limited in amount as described above, the rats lost weight but maintained a general appearance of health. The egg counts during the periods of partial starvation did not decline appreciably.

Figure 1 illustrates typical weight and egg count variations for two rats, D12 and D15, one in each of the two groups described above. The variations in the total numbers of eggs eliminated by rats of the two groups are graphically represented in figure 2.
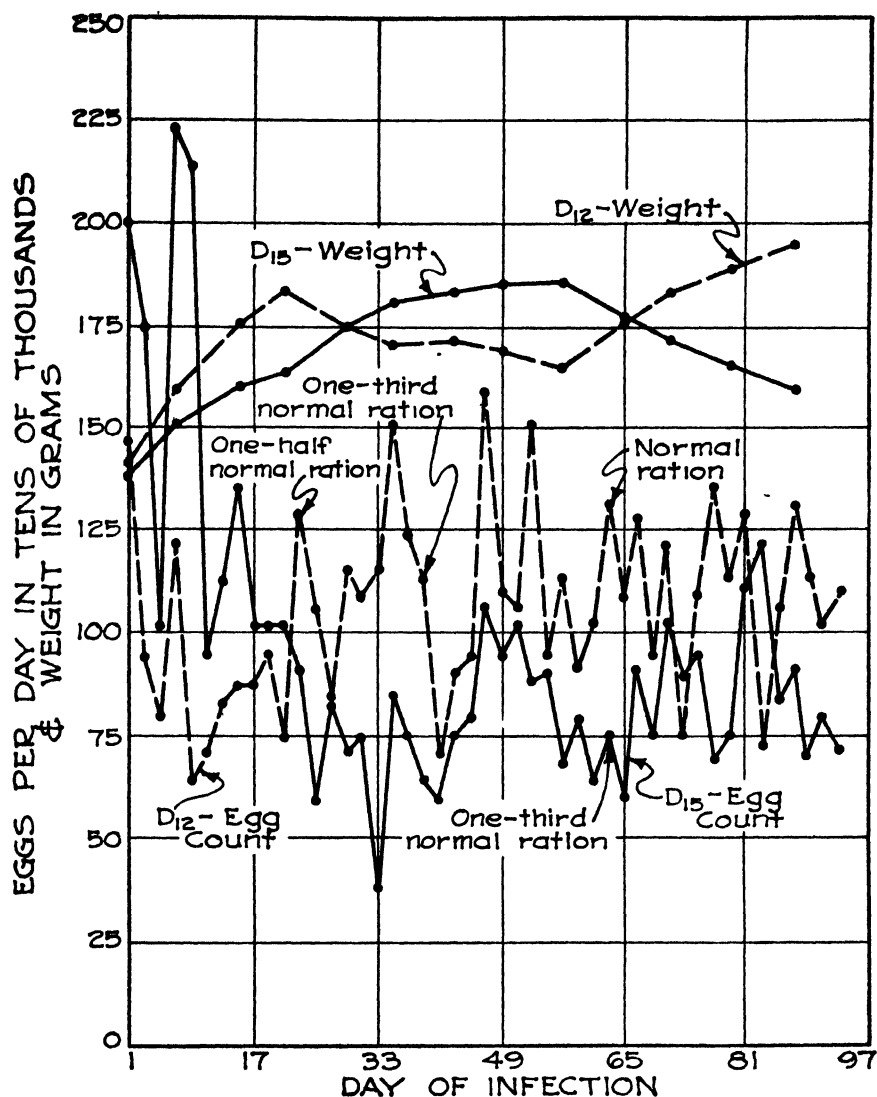
Fig. 1. Variations in body weight and numbers of eggs eliminated for rats D12 and D15 with free and limited food intake.

DISCUSSION

A rather thorough search of the literature has not revealed that the confused flour beetle *Tribolium confusum* has ever before been recorded as a natural or experimental host for the rat tapeworm *Hymenolepis diminuta* (5, 6, 20, 21, 22, 23, 24, 28, 29, 30, 31, 32, 37, 38, 39, 43, 47, 48). It is not surprising, however that the cysticercoid should be able to de-
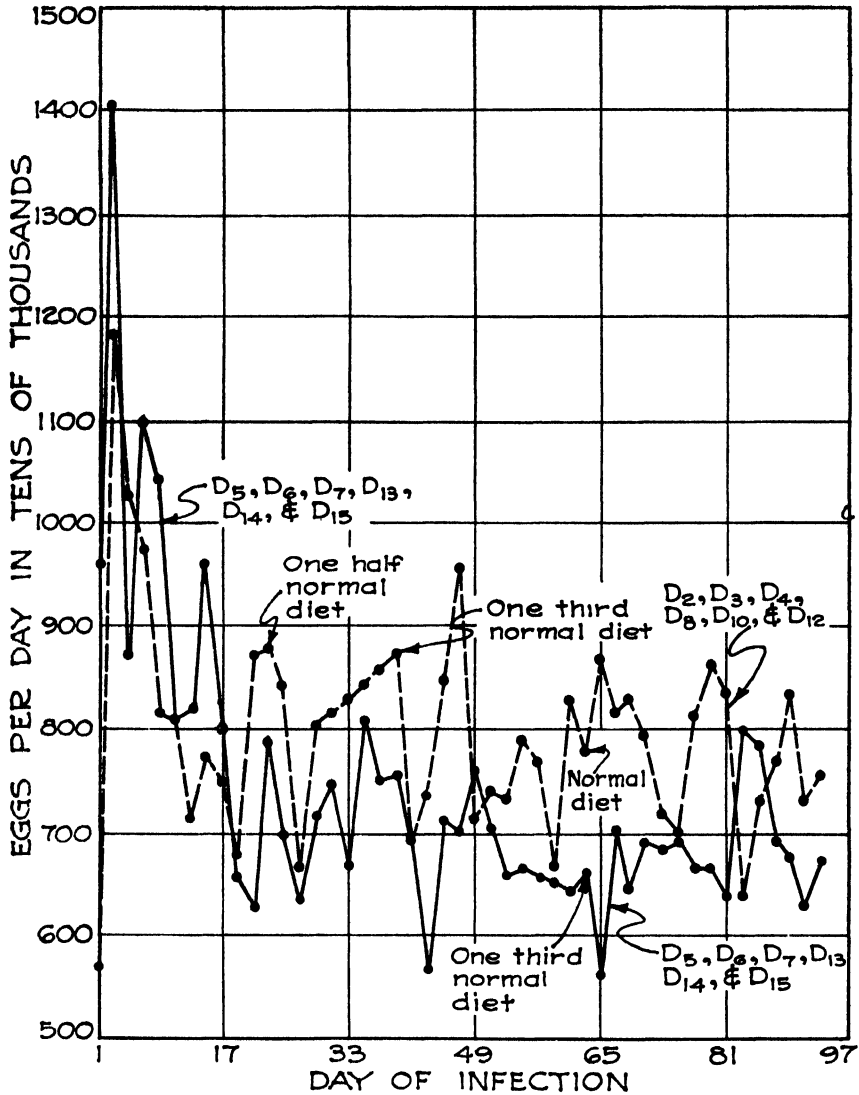
Fig. 2. Comparison of the total number of tapeworm eggs eliminated daily by the group of rats D2, D3, D4, D8, D10 and D12, and the group D5, D6, D7, D13, D14 and D15, with free and limited food intake.

velop in this insect, since it is known to occur in a large number of arthropods and has been reported for the allied species *Tribolium castaneum* (28, 29).

In the present investigation only the adult beetles were used as vectors. Because of the grain-infesting habit of this insect, it seems probable that it might serve as a natural as well as an experimental host. The suc-

cessful infecting of rats by means of confused flour beetles three weeks after the ingestion of eggs by the insects, suggests that the time required for the development of the cysticercoid in this host is comparable to that found by Joyeux to obtain in other hosts (31).

It is evident from the graphic representations of results that there is a considerable day to day variation in the number of eggs present in the fecal material. This is not unexpected, since varying numbers of segments are separated from the major part of the worm from time to time and the parenchyma partially digested. This day to day variation made it necessary that egg counts should be continued for each rat over a fairly long period of time and that the general trend of the counts should be considered rather than that an attempt should be made to account for each rise or fall.

It may be noted that a high initial production of eggs is characteristic of *Hymenolepis diminuta* infections in the rat. There follows more or less of a leveling off with somewhat lower counts. Although it may be possible that this is due to the development of some immunity on the part of the host, it is questionable whether this can be considered to be the case with such a parasite as *Hymenolepis dimiuuta* which lives in the lumen and is not a tissue dweller. Furthermore, there is no evidence of a continuous development of immunity since the numbers of eggs eliminated did not continue to decline beyond the first week or two of the infection.

CONCLUSIONS

1. The adult flour beetle *Tribolium confusum* may serve as a vector for the rat tapeworm *Hymenolepis diminuta*.

2. The cysticercoids developing in the flour beetle are infective to the rat after approximately three weeks in the intermediate host.

3. Partial starvation of the host by restriction of the food intake to one-third the normal amount does not cause a decrease in the numbers of tapeworm eggs eliminated.

4. A high initial egg production is characteristic of *Hymenolepis diminuta* infections in the rat, followed by a more constant rate of development at a somewhat lower level.

II. DEFICIENCY OF VITAMINS B_1 AND G

A differentiatial effect on intestinal parasites of a diet high in yeast has been reported by Hegner (25) who found that such a diet fed to rats resulted in an increase in the numbers of *Trichomonas muris* and *Hexamitus muris*, but in a decrease in the numbers of *Giardia muris*. Allen (3) reported that chickens harboring *Eimeria tenella* eliminated fewer oocysts for the first five days of the patent period when given a high protein, high vitamin diet than did chickens on a low protein, low vitamin diet. After the fifth day, however, the numbers eliminated by the former group became and remained relatively higher and there was chronic coccidiosis. Becker and Morehouse (11) found that yeast appeared to contain a

factor favorable to the growth of *Eimeria miyairii* in the rat, and that the numbers of oocysts were greatly reduced when there was a deciency of vitamins B_1 and G (14). Deficiency of vitamin B_1 alone did not appear to alter the number of oocysts developed, but the vitamin G deficient rats eliminated significantly fewer oocysts than their controls. There was not, however, so great a reduction as in those receiving neither vitamin (12). Later the same authors (13) stated that the factor which promoted growth in coccidia appeared to be linked with the vitamin G complex since wheat germ and other substances furnished it as well as yeast. Liver as a source of the vitamin did not favor the parasite (15). More recently (17) they reported that rats eliminated fewer oocysts when rice polishings, powdered liver or skim milk, in that order, were used as a source of vitamin G, than when the vitamin was supplied by yeast. Vitamin G or some factor associated with it was considered by Becker and Derbyshire to promote growth of coccidia (18, 19).

The effects of dietary modications on helminth parasites also have received attention. Vitamin B_1 was reported by Zimmerman, Vincent and Ackert (49) to be a factor in the resistance of chickens to *Ascaridia lineata*. The worms in the intestines of the chickens on a diet deficient in vitamin B_1, but adequate in other respects, were larger and more numerous than those in chicks of the same age receiving an adequate diet. Ackert and Nolf (1) suggested that yeast may contain a factor favoring the growth of *Ascaridia lineata* in chickens. Ackert (2), however, did not indicate that there is any evidence that this nematode requires vitamin B_1, and, with Beach (8), was not able to demonstrate the presence in yeast of any special growth factor for this parasite.

DEFICIENCY OF VITAMINS B_1 AND G

For the investigation here described, in order to observe the effect upon the tapeworm *Hymenolepis diminuta* of a diet deficient in both vitamins B_1 and G, four young rats (designated as A1, A2, A3, and A4) were parasitized with this cestode in the manner described in Part I; they were maintained on the same control diet and the counts of eggs eliminated per day by each animal were obtained in the same way.

After egg counts had been made for a control period, two of the rats were given a ration similar to that described before, but with yeast omitted and ten per cent more sugar. This diet is comparable to those frequently used for vitamins B_1 and G deficiency. On this diet the rats declined rapidly both in weight and in general appearance of health, and the numbers of worm eggs also declined, disappearing completely from the droppings of one of the hosts. After being restored to the control diet, the rats improved in weight and appearance and the numbers of eliminated eggs mounted. When the other two rats were given the deficient diet, eggs disappeared from the droppings of both and no tapeworms could be found in the intestines at autopsy.

The results obtained for the rat designated as A2 may be taken as an
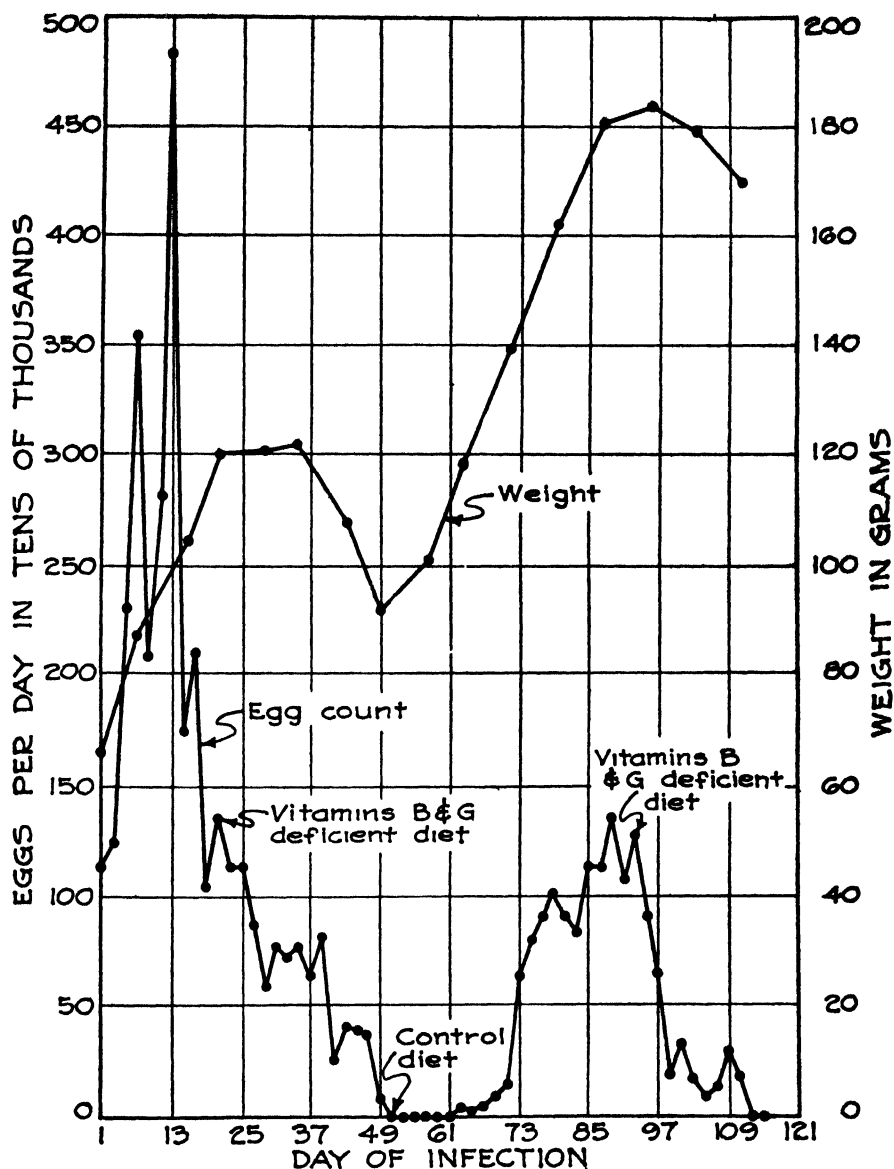
Fig. 3. Variations in the numbers of tapeworm eggs eliminated daily by rat A2 alternately on a control diet and one lacking in vitamins B₁ and G.

example and are represented in figure 3. This host was first maintained on the control diet, then on the experimental ration, restored to the control diet for a time, and then again given the ration lacking in yeast. The variations in the egg counts and in the body weight of the host are indicated in the figure.

Deficiency in Vitamin B₁. In order to observe the effects of a diet deficient only in vitamin B_1, four young rats harboring rat tapeworms were maintained on the control diet described in the foregoing account for a preliminary period of 45 days, during which egg counts were made every two days. Three of the four were then given a ration similar in all respects to the control diet except that the yeast used had been autoclaved for two hours at 120°C. This diet is not adequate for vitamin B_1. Because
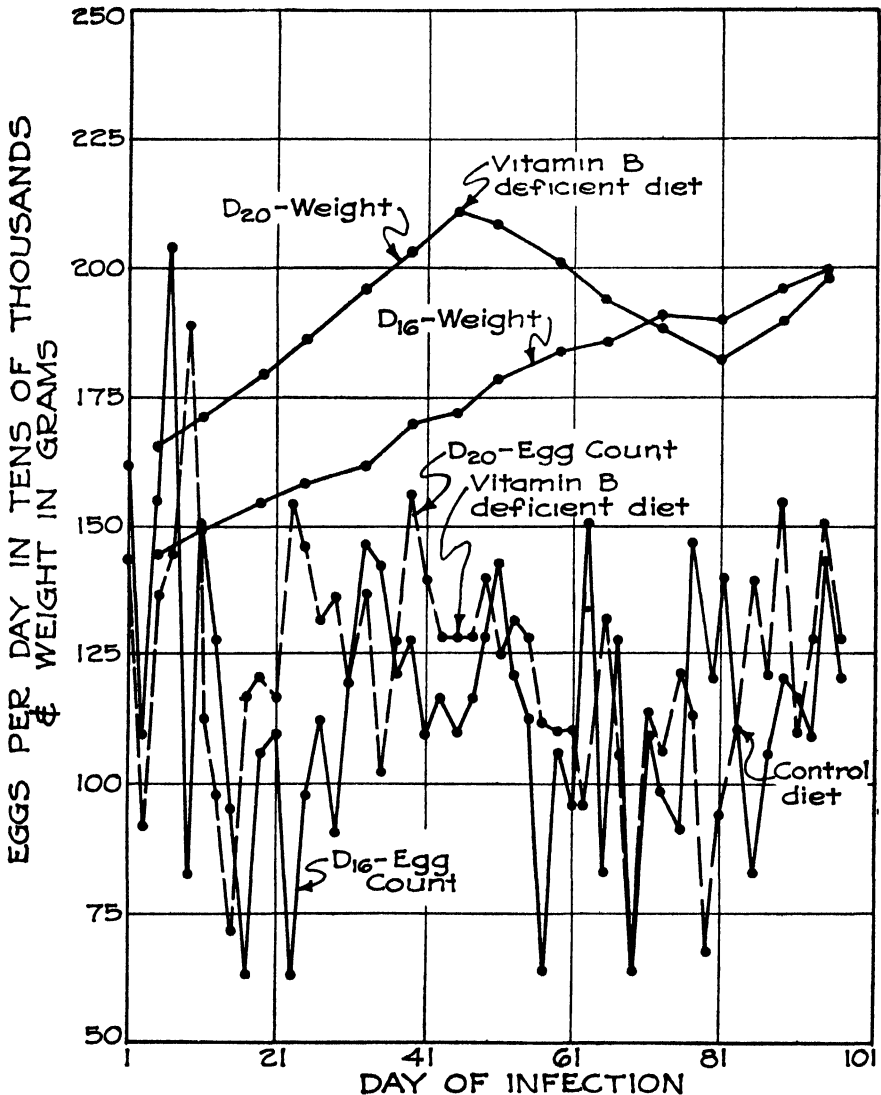
Fig. 4. Variations in body weight and numbers of tapeworm eggs eliminated daily by rat D16 on a control diet and by rat D20, alternately on a control and a vitamin B₁ deficient diet.[4]

the numbers of eggs passed were maintained at the previous level for two of these experimental rats, but declined somewhat in one case, it was considered desirable to try the effect of this diet a second time.

Seven young rats were fed flour beetles exposed to infection with *Hymenolepis diminuta* in the manner described. In the droppings of all but one, eggs were found in from 19 to 23 days. The six young infected rats were fed the control diet containing unautoclaved yeast, and deter-
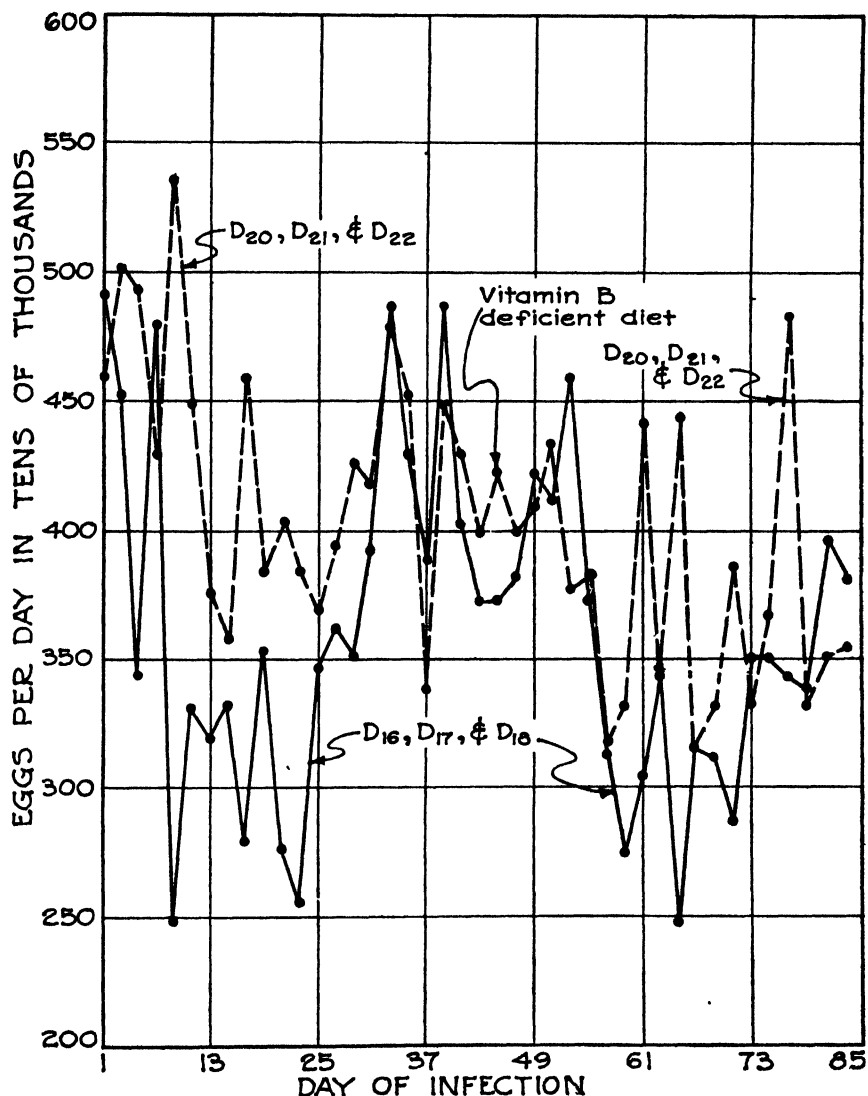
Fig. 5. Comparison of the total numbers of tapeworm eggs eliminated by rats D16, D17 and D18 maintained on a control diet and by rats D20, D21 and D22 maintained alternately on a control and a vitamin B₁ deficient diet.

minations of the numbers of eggs eliminatd were made for a control period of 45 days. At that time the three rats, D20, D21 and D22 were given the diet in which the autoclaved yeast was substituted, whereas the other three, D16, D17 and D18 were continued on the control diet.

The animals on the experimental diet ceased making normal gains and after several weeks fell off sharply in body weight. There was, however, no marked decreased in the numbers of eggs eliminated during this time as compared to those passed during the 45-day control period.

On the eighty-fourth day, one of the experimental animals, D20, was restored to the control diet. This animal made gains in weight, but did not show a notable increase in the numbers of worm eggs passed.

The variations in the weights and numbers of eliminated eggs for the two rats D16 and D20 are shown in figure 4. Rat D16 was maintained continuously on the control diet; rat D20 was shifted to the diet deficient in vitamin B_1 and then again to the control ration.

The total numbers of eggs eliminated by rats D16, D17 and D18 are compared graphically with those passed by rats D20, D21 and D22 in figure 5. Until the forty-fifth day, rats in both groups were given the same diet, the control ration containing unautoclaved yeast. At that time, autoclaved yeast replaced the unautoclaved for one group. This graphic representation is continued only until the eighty-third day, although egg counts were made for an additional two weeks, because after that day the three rats D20, D21 and D22 could not be grouped together, as D20 was again given the control ration.

Effects of a Diet Deficient in Vitamin G. Eight young male rats were fed flour beetles which had been given gravid proglottids of *Hymenolepis diminuta* and all eight became infected. They were maintained on the same control diet, containing unautoclaved yeast, described above. After a month, during which the numbers of eggs being passed daily by each rat were determined, four of the rats, C1, C2, C3 and C4, were transferred to the diet containing no yeast, and consequently deficient in both vitamins B_1 and G. Vitamin B_1 was supplied by the daily administration of four drops of tiki tiki[5] to each experimental rat. This was readily taken from a pipette by those animals whose diet lacked yeast.

The rats on the experimental diet lost weight although they continued to eat fairly well. The skin became coarse; the hair, matted and unkempt. On the one hundred thirty-ninth day of the infection, two of the experimental rats were again given the control diet. These animals regained weight and a general appearance of health and tidiness.

The numbers of eggs passed by rats maintained on the vitamin G deficient diet were greatly decreased. Typical results are presented graphically in figure 6. Rat C1 was fed the control ration for the first month of the infection, transferred to the experimental food for three and a half months and again placed on the control ration for a month. Rat C4 was transferred to the experimental ration for the duration of the experiment

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[5] Obtained from the Bureau of Science, Manila, Philippine Islands.
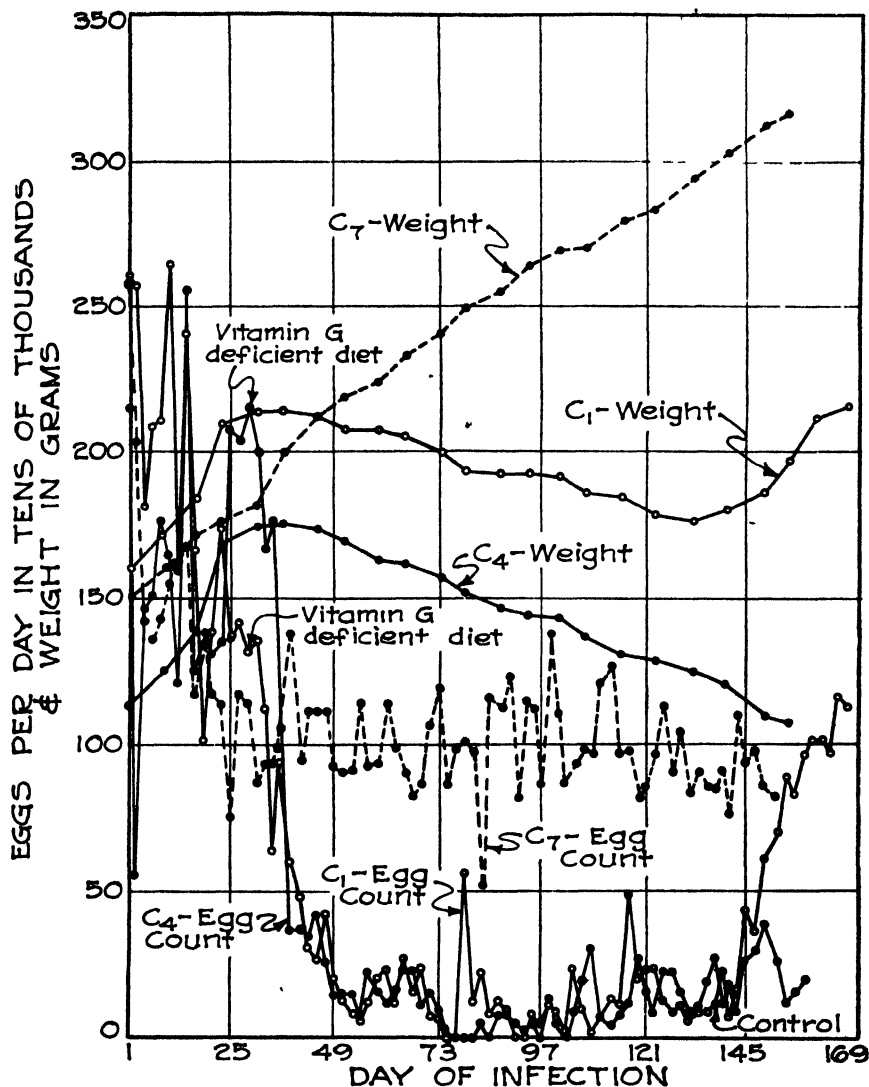
Fig. 6. Variations in body weight and numbers of tapeworm eggs eliminated daily for rats C1, C4 and C7 on vitamin G deficient and control diets.

after being given the control ration for the first month of infection. Rat C7 was maintained for the entire period on the control diet. Both the egg counts and the body weights of the hosts are represented in the figure.

Figure 7 compares graphically the total numbers of eggs passed by, rats C1, C2, C3 and C4 which were transferred to the experimental diet, and those eliminated by rats C5, C6, C7, and C8 which continued on the control diet. The figure indicates the course of the infection from the first to the one hundred thirty-ninth day.
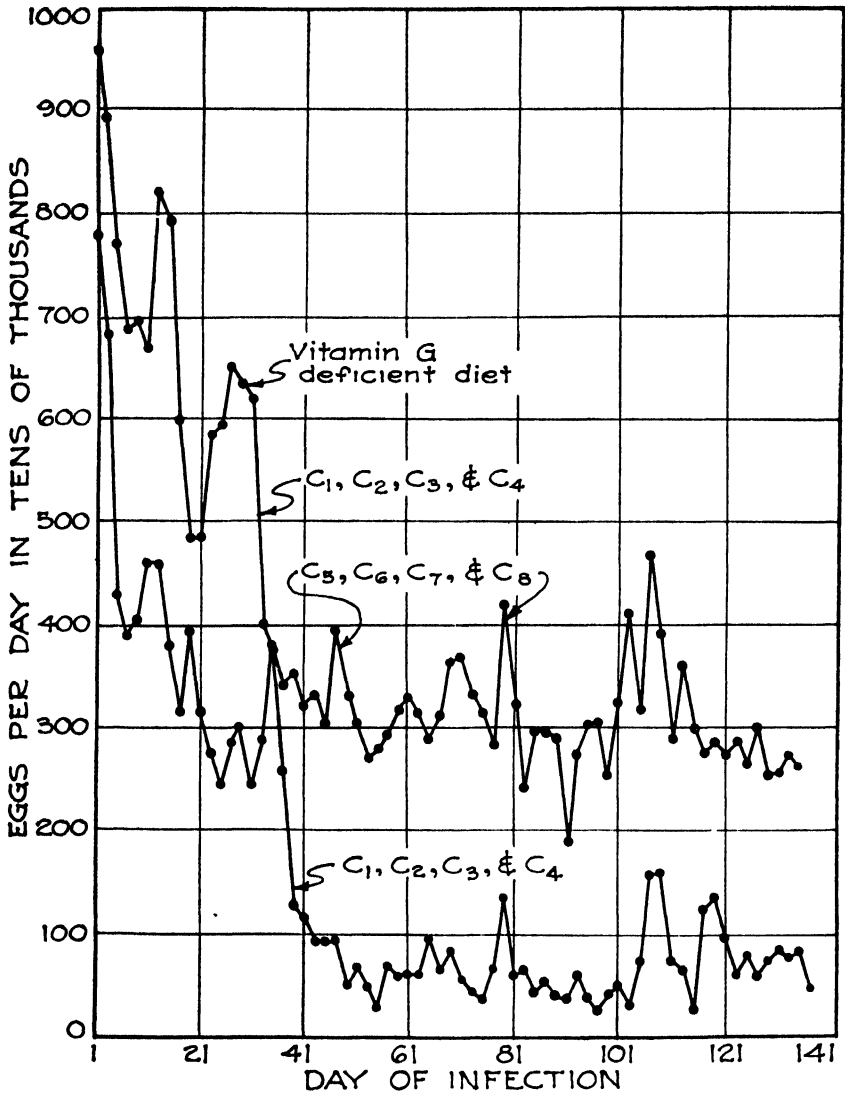
Fig. 7. Comparison of the total numbers of tapeworm eggs eliminated by rats C5, C6, C7 and C8 maintained on the control diet and by rats C1, C2, C3 and C4 given alternately a control and a vitamin G deficient diet.

DISCUSSION

That it is possible to influence the reproductive processes of the rat tapeworm by the diet of the host seems evidenced by the observations described. There appears to be some factor present in yeast which is required for maximum reproductivity of the rat tapeworm. The fact that the production of eggs did not decrease when the yeast had been auto-

claved (see figures 4 and 5) suggests that this factor is not the labile vitamin B_1. The decline of reproductive power of the parasite when the host was maintained on a vitamin G deficient diet, however, indicates that it may be associated with the vitamin G complex from some sources. It cannot be stated that this factor is identical with vitamin G.

It has been suggested to the author that the decrease in numbers of eggs eliminated might be merely a secondary result of the decreased amount of fecal material because of less residue from lowered food intake on the part of the host. However, since the rats were continually kept over pans so that all of the pellets passed were collected, and since the determinations were made, not on the basis of numbers of eggs per unit weight of feces, but on numbers of eggs eliminatd over a two-day period, it would seem that all the eggs produced would be accounted for, whether they occurred in a small number of hard pellets or in a larger number of bulky ones.

Furthermore, this suggestion would not explain why egg counts declined on the vitamin G deficient diet which was associated with loss of body weight on the part of the hosts, but were not decreased on the vitamin B_1 deficient diet, also associated with decreased food intake and loss of weight. Nor would it account for the continued collection of large numbers of eggs when the food intake was limited to one-third the usual amount (see Part I).

CONCLUSIONS

1. It is possible to influence the numbers of eggs produced by the rat tapeworm *Hymenolepis diminuta* through modification of the host's diet.

2. A diet deficient in both vitamins B_1 and G is associated with a decrease in egg output.

3. This effect is apparently not due to the labile vitamin B_1, as a diet deficient in this vitamin is not associated with a lowered production of eggs.

4. There appears to be a factor associated with the vitamin G complex which is necessary for normal egg production by the rat tapeworm. A diet lacking in vitamin G does not furnish this factor. It is not maintained that the factor is identical wtih vitamin G.

5. It is suggested that the effects of the various diets are probably produced directly upon the parasite rather than indirectly through effects on the hosts, since certain diets detrimental to the hosts are not associated with lowered egg production on the part of the parasite, whereas other diets appear to be deleterious to both host and parasite.

III. MILK, SOY BEAN OIL MEAL, WHEAT MIDDLINGS

The maintenance of rats harboring *Giardia muris*, *Trichomonas muris* and *Hexamitus muris* upon an exclusive milk diet was reported by Hegner (25) to be associated with a decrease in the incidence of all three forms. Ratcliffe (41) found a reduction in the numbers of trichomonads

in the ceca of rats fed a diet high in protein in the form of casein. Ratcliffe noted also (42) that numbers of *Trichomonas hominis* and *Pentatricho-monas ardin delteili* became lighter when the host rats were given a diet which increased intestinal acidity and still lighter on a high protein diet containing 56 per cent unpurified casein. The organisms were not elimi-nated, however, by the use of this diet. In rats and in monkeys, Kessel (33) reported that an exclusive milk diet or neutral diets with lactic acid or large amounts of lactose added, produced a lowering of the pH of the colon contents and a decrease in the amoebae and trichomonads present. Kessel and K'E-Kang (34) fed an exclusive raw milk diet to monkeys and to children and observed that during that period there was almost always a reduction in the numbers of intestinal amoebae, and in some cases the intestine was cleared of *Endamoebae dysenteriae* and other forms. Infections of *Balantidium coli* from the pig were established in rats by Schumaker (45). Within a week the infection was greatly re-duced or eliminated from rats on a diet of whole milk. Diets high in casein prevented infection and eliminated heavy infections within eight days, but a high carbohydrate diet containing no casein was favorable for the de-velopment of the parasite.

Beach and Davis (7) found that chicks were given an appreciable protection against the effects of coccidiosis by being fed mash containing 40 per cent dry skim milk and 20 per cent lactose. The effect was attrib-uted by the authors to the greater acidity of the cecal contents. Their conclusions were drawn only from observations of the birds, and no counts of oocysts were made.

Becker and Morehouse (10) did not find the addition of skim milk or of lactose to the diet of rats to exert any restraining influence upon the numerical increase of *Eimeria miyairii*, but when the diet was high in casein, the numbers of oocysts were somewhat reduced (14). Rats elimi-nated fewer oocysts when skim milk was used as a source of vitamin G, than when the vitamin was supplied by yeast (17).

Certain constituents of diets for chicks, particularly skim milk, butter-milk and wheat middlings in certain amounts or combinations, were re-ported by Becker (16) to increase the severity of *Eimeria tenella* infec-tions in chicks. Becker and Derbyshire (18) found no correlation be-tween the bulkiness of the ration and the number of oocysts of *Eimeria miyairii* eliminated by rats, nor between the growth of host and parasite development. They found the ratio of coccidium-growth-promoting sub-stance in soy bean oil meal as compared to yeast to be 0.51; and in wheat flour middlings, 2.65 (19).

Reports of relations of diets to cestode parasites are not numerous. Becker (9) gave only whole milk (plus copper sulfate and iron chloride for seven of the hosts) to 15 rats, all passing eggs of *Hymenolepis nana*, and kept on screen to prevent coprophagy. After five and one-half days, no eggs could be found by smear or flotation, nor were eggs passed subse-quently during the 30 days that the rats were on a milk diet. After this

time the rats were again given a grain diet, and eggs reappeared in the feces of 11. No tapeworms were found in the others. Milk apparently produced a cessation of egg production by the parasite, but not death.

THE EFFECTS OF AN EXCLUSIVE RAW MILK DIET

In the investigation here reported, six young rats maintained on Steenbock's growing ration (yellow corn meal, 71 per cent; linseed oil
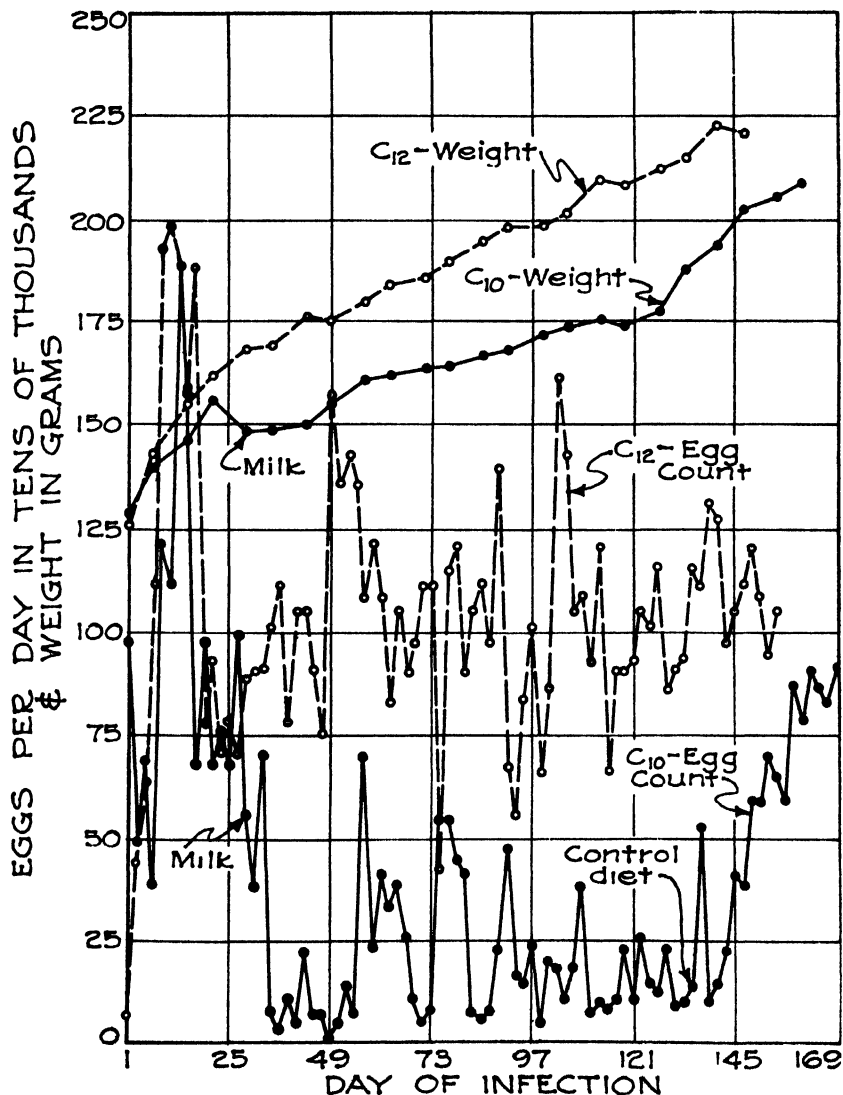
Fig. 8. Variations in body weight and numbers of tapeworm eggs eliminated daily for rat C12, on Steenbock's ration, and rat C10, alternately on Steenbock's ration and milk.
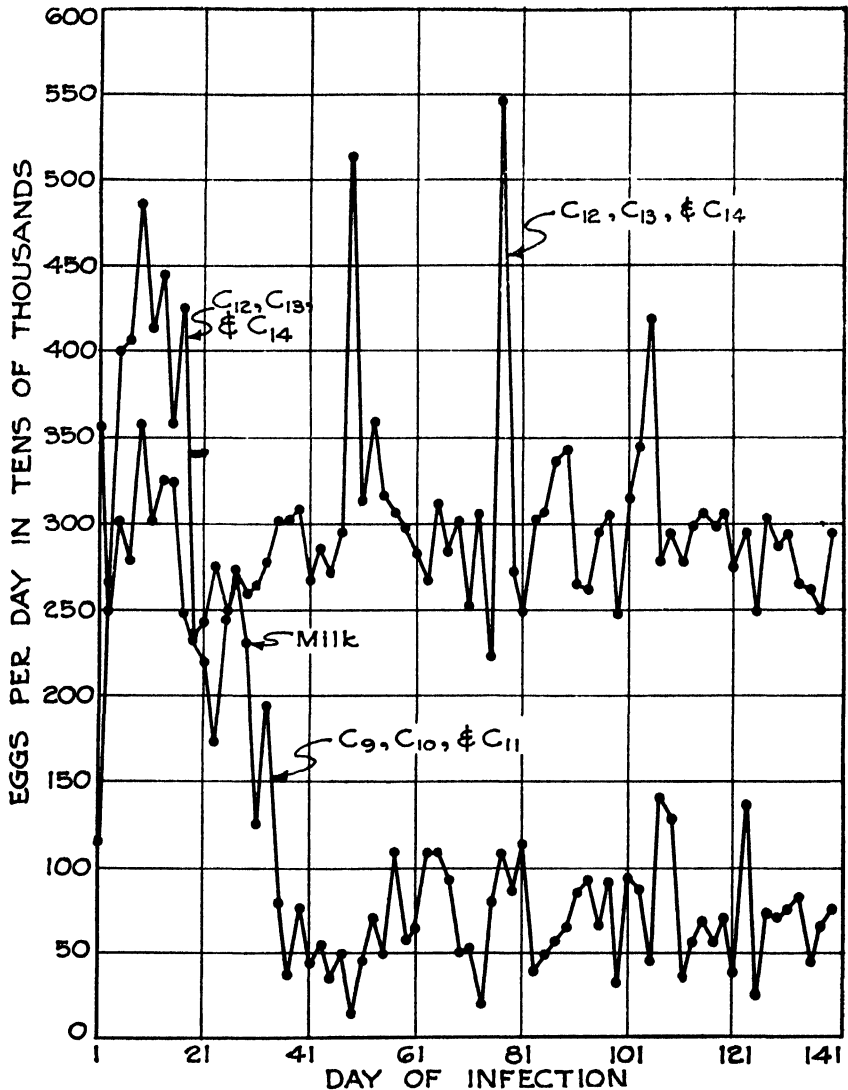
Fig. 9. Comparison of the total numbers of tapeworm eggs eliminated daily by rats C12, C13 and C14 on Steenbock's ration and by rats C9, C10 and C11, alternately on Steenbock's ration and milk.

meal, 16 per cent; ground alfalfa, 2 per cent; sodium chloride, 0.5 per cent; calcium carbonate, 0.5 per cent; dried skim milk, 10 per cent) were fed infected flour beetles[6] and all began to pass eggs of *Hymenolepis diminuta* in from 19 to 25 days. The numbers eliminated each day by each rat were determined. On the thirtieth day after the first occurrence

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[6] According to the method described in Part I.
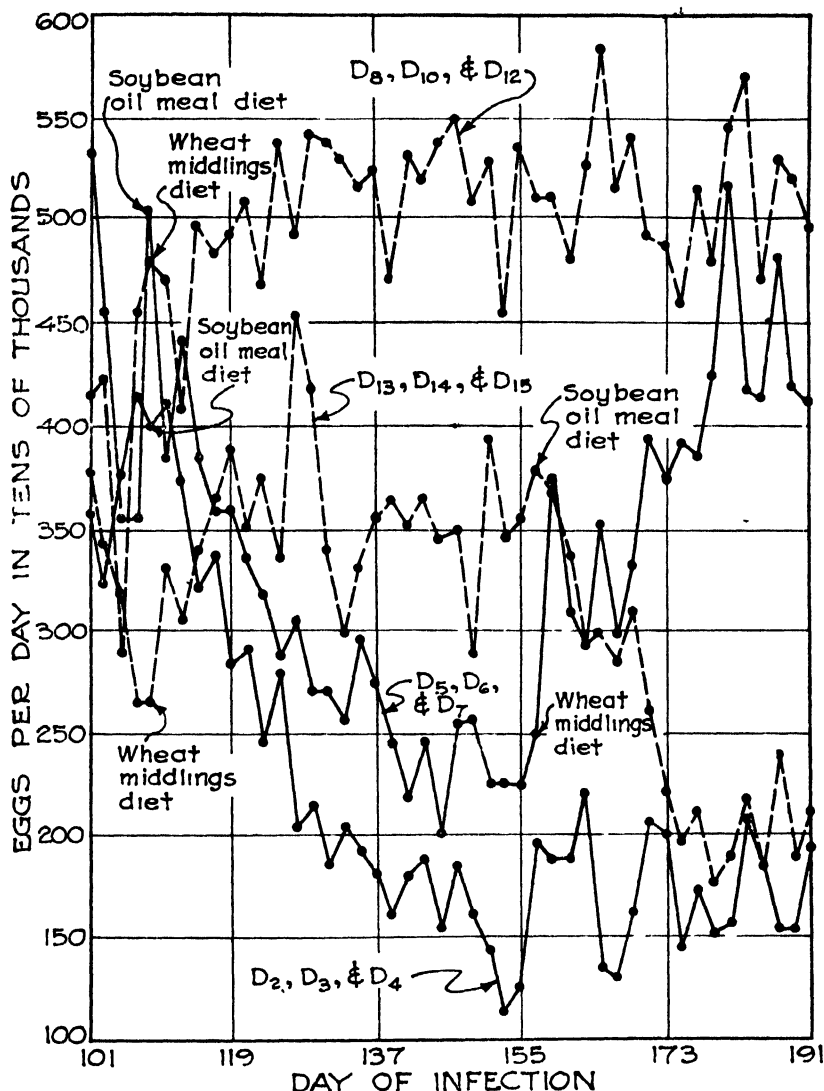
Fig. 10. Total numbers of tapeworm eggs eliminated daily by the group of rats D2, D3 and D4; the Group D5, D6 and D7; the group D8, D10 and D12, and the group D13, D14 and 15 maintained on diets containing soy bean oil meal or wheat middlings.

of eggs in the pellets, three of the rats, C9, C10 and C11, were taken off the growing ration and given only whole raw milk. The other three rats were maintained on the growing ration as before. Egg counts were made over a period of four months. On the one hundred thirty-fifth day of the infection, one rat, C10, was restored to the growing ration, but rats C9 and C11 were continued on milk. The egg counts for C10, typical of the results for the experimental rats, and for C12, representative of the con-

trols, are shown graphically in figure 8. The weight variations of the two rats are indicated in the same figure.

Figure 9 shows a graphic comparison of total numbers of eggs eliminated each day by the three experimental rats, C9, C10 and C11, with those passed by the three controls, C12, C13 and C14, from the first to the one hundred thirty-fifth day. The three experimental rats cannot be grouped subsequent to that day, as they no longer all received the same diet.

COMPARISON OF THE EFFECTS OF SOY BEAN OIL MEAL AND WHEAT MIDDLINGS IN THE DIET

Twelve young rats harboring rat tapeworms were divided into two groups of six rats each. The rats in one group were given a modification of the control diet in which 10 per cent soy bean oil meal was substituted for the 10 per cent yeast; the other, a diet in which 30 per cent wheat middlings was substituted for the 10 per cent yeast and 20 per cent of the sugar.

After 46 days on these rations, three of the rats in each group were shifted to the alternate diet; the other three were allowed to continue as before.

The rats continued to gain on both rations at approximately the same rate. The numbers of eggs eliminated, however, were appreciably higher when the modified diet included wheat middlings than when soy bean oil meal was substituted, as may be seen in figure 10. This figure represents graphically the total numbers of eggs eliminated by rats D2, D3 and D4 which were maintained on the soy bean oil meal diet for the course of the experiment, and the numbers passed by rats D5, D6 and D7 which were first maintained on the soy bean oil meal ration and then shifted to that containing wheat middlings. Figure 3 also shows the total counts for D8, D10 and D12 on the wheat middlings diet, and for D13, D14 and D15 first on wheat middlings and then on soy bean oil meal. In this way comparisons may be made between the results obtained for those rats on each of the diets for the entire period of observation, and for those transferred from one diet to the other.

CONCLUSIONS

It has been previously suggested that there is some factor present in yeast and associated with the vitamin G complex which is necessary for maximum reproductivity of the rat tapeworm. (See Part II of this series.) Raw milk appears to furnish an insufficient amount of this factor to keep the worm at its maximum reproductive level; wheat middlings seem to furnish more than does soy bean oil meal, although this latter supplement does not lack it entirely.

IV. OBSERVATIONS ON WORM BURDENS

In the previous papers of this series[7], procedures were described in which the rat hosts were maintained on various diets (Steenbock's grow-

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[7] Parts I, II, and III.   '

ing ration, raw milk, diets adequate and deficient in both vitamins B_1 and G and in each of these vitamins separately, and diets containing soy bean oil meal or wheat middlings), and the effects on the reproductivity of the cestode parasite *Hymenolepsis diminuta* were noted. In the planning of these experiments it was considered necessary to have a control period for each rat so that during the experimental period its elimination of eggs could be compared to its previous record in that respect. The egg counts for one rat could not be directly compared with those from another since there was no way of assuring that they should be infected with the same number of worms.

When, at the conclusion of any of the investigations referred to above, the rats were killed with illuminating gas and the intestines opened, the tapeworms present were removed ánd counted. The parasites were allowed to relax for one hour in cold water and then were measured.

RELATION OF THE WORM BURDEN TO THE NUMBERS OF EGGS ELIMINATED

For those rats (numbering 26 altogether) in the various experiments which were maintained on the ration designated as the control diet, for the first month of infection, it was possible at autopsy to obtain data on the relationship of the egg counts to the numbers of tapeworms harbored. There was revealed no direct correlation between the numbers of eggs found in the droppings and the worm burden, but rather it appeared that the number of eggs produced by a worm was influenced to some extent by the number of parasites present, so that a rat harboring a considerable number of worms might pass no more eggs than one harvoring only a few or even a single parasite, as shown in table 1. In this table, those rats found to have harbored the same number of parasites are grouped, and the average number of eggs eliminated per day by each rat during the first month of infection noted, together with the mean for each group. The average number of eggs produced per day for each worm was calculated and also appears in the table. During the period involved, all of these rats had similar adequate diets.

These results are represented graphically in figure 11 which shows the average number of eggs passed per day per rat for each group harboring different numbers of parasites, and in figure 12, which represents the average number of eggs produced per day per worm for the same groups.

RELATION OF THE WORM BURDEN TO THE LENGTHS OF WORMS HARBORED

In table 2, all of the 32 rats used in the investigations previously described, regardless of diet before autopsy, are arranged in groups in an ascending series according to the numbers of tapeworms harbored. The average length of the parasites in each host is recorded, together with the diet which that host had been receiving prior to its death. The "control" diet is the adequate, complete food mixture described in Part I and designated as such throughout this series of papers; the diet indicated as "Steenbock's" is Steenbock's growing ration (Part III); "milk"

TABLE 1· *Mean numbers of tapeworm eggs eliminated per day per rat and per day per worm for rats on an adequate control diet, but harboring different numbers of parasites*

Number of worms harbored	Rat	Average number of eggs (10^3) eliminated per day for first month of infection	Average number of eggs (10^3) per worm per day
1.	D8	1777	1777
1.	C7	1439	1439
1.	D19	1246	1246
1.	D3	1233	1233
1.	D2	978	978
1.	D12	940	940
1.	C5	430	430
		(Mean: 1149)	(Mean: 1149)
2.	D4	1478	739
2.	D5	1460	730
2.	D15	1378	689
2.	D16	1284	642
2.	D13	994	497
		(Mean: 1319)	(Mean: 659)
3.	D22	1359	453
3.	D17	782	294
		(Mean: 1070)	(Mean: 373)
4.	D6	1684	421
4.	C8	1520	380
		(Mean: 1602)	(Mean: 400)
5.	D21	1735	347
6.	C1	1848	308
7.	D10	1950	280
7.	D7	1715	245
		(Mean: 1832)	(Mean: 262)
8.	D14	1808	226
10.	C3	1790	179
11.	C4	1595	145
12.	D18	1404	117
14.	C2	1694	121
42.	C6	672	16

refers to an exclusive whole raw milk diet; "soy bean oil meal" and "wheat middlings", to the diets including these supplements (Part III).

DISCUSSION

For several reasons, it seems probable that all the worms harbored by any one rat were received at the same time. The infected beetles were kept in glass jars with tight screw caps to prevent their escape, and the immediate dropping of the rat pellets through the bottoms of the cages into the trisodium phosphate solution in the pans makes it unlikely that any other insects would have been accidentally infected. It might be
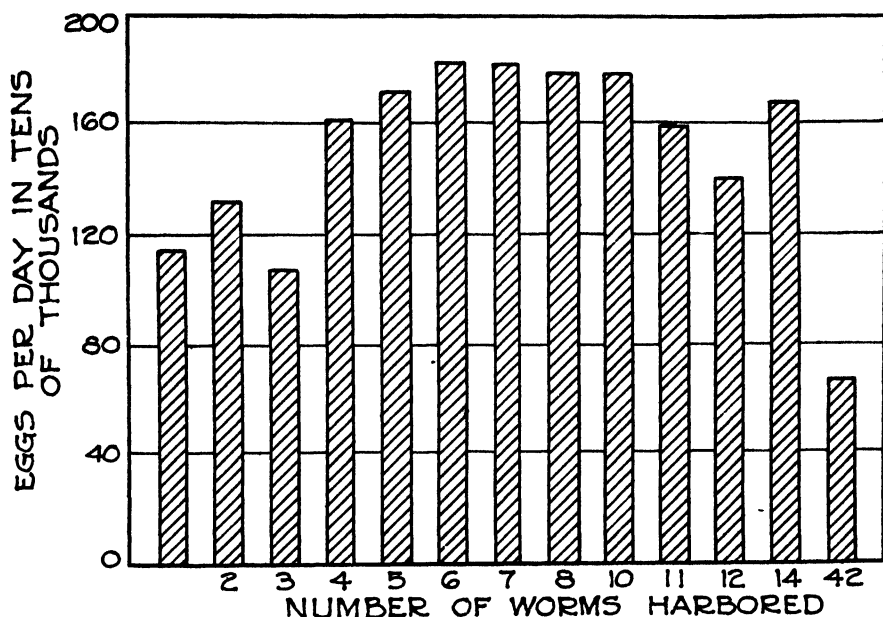
Fig. 11. Mean numbers of tapeworm eggs eliminated per day by rats harboring various numbers of worms.

noted in this connection that from time to time uninfected rats were kept for considerable periods in the same laboratory and none of them were ever found to have become hosts for the rat tapeworm, although the droppings were examined periodically. Moreover, Palais (40) reports his inability to infect rats with additional rat tapeworms once an infection was established. On the other hand, no evidence was ever seen in the pans of spontaneous loss of a worm. Series of proglottids were sometimes
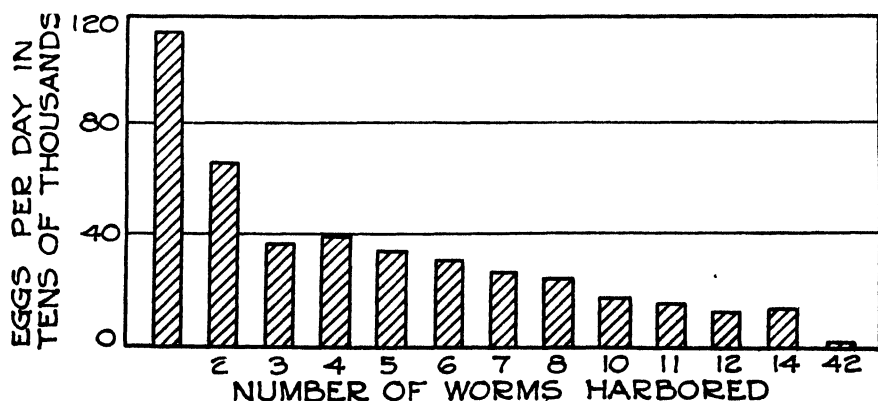
Fig. 12. Mean numbers of eggs produced per worm per day by worms grouped according to the numbers of worms present.

present in the droppings, but these were always gravid proglottids from the posterior portion of the worm. It seems, therefore, that during the course of any investigation, there was no change in the number of worms present in any one host and that the variations in the egg counts represent actual variations in the numbers of eggs being developed rather than changes in the worm burden of the host.

It was not possible to compare results on a per worm basis, since the numbers of eggs produced per worm are not the same for different severities of infection (see figure 12). Only the general trend of one egg count curve may legitimately be compared with that of another.

Reference to table 1 and to figure 11 will show that there was little difference in the average numbers of eggs eliminated by rats harboring one, two or three tapeworms, and rats harboring from four to fourteen worms eliminated very nearly the same average numbers. A single helminth parasite of this type harbored in an intestine would seem to have optimum conditions for reproduction and the numbers of eggs developed by any worm decrease fairly regularly as the worm burden increases (see figure 12).

Table 2 illustrates the tendency for the parasites to be smaller as the worm burden increases. When as many as 42 worms were harbored, some remained extremely small, and examination by microscope did not reveal any eggs whatever. Young and mature proglottids were present but no gravid segments, and the width of the worms was less than the width of corresponding portions of the larger worms. The differences in size of parasites as well as differences in the numbers of eggs produced in infections of differing severity might conceivably be due to competition for food or to crowding. If the decreased amount of food available for each parasite were the determining factor, it would be expected that limiting the food intake of the host would have shown similar results. Yet, reducing the amount of food ingested by the host to one-half or even to one-third the normal amount produced no apparent decrease in reproductive rate or power (See Part I), whereas worms dwelling in an intestine with one other worm produced only 57 per cent (See table 1) as many eggs as those worms which were the sole residents.

If the results observed be ascribed to crowding, the effects might have been produced by the actual pressure and lack of room for growth. Indeed, when many worms are harbored the intestine gives the impression of being so full of parasites as to be nearly obstructed. The decreased opportunity for absorption when many worms are crowded together may be a factor as may also a detrimental effect of the greater amount of excretory products present from the many parasites.

A decrease in reproductive power associated with an increased parasite population has been noted by other investigators. Hill (27) reports a decrease in the number of eggs produced per hookworm as the number of worms harbored by the human host increased. In the case of the dog hookworm, Sarles (44) found a smaller egg production per worm in

TABLE 2. *Mean lengths of tapeworms found in rats harboring different numbers of worms*

Number worms Present	Rat	Average length of tapeworms (cm.)	Diet of host before death
1	D20	92	Control
1	D21	91	Soy bean oil meal
1	C5	89	Control
1	D8	88	Wheat middlings
1	C7	78	Control
1	D12	76	Wheat middlings
1	D3	71	Soy bean oil meal
		(Mean: 83.6)	
2	D16	86.5	Control
2	C10	74	Steenbock's
2	D15	71.5	Soy bean oil meal
2	D13	69.5	Soy bean oil meal
2	D4	58	Soy bean oil meal
2	D5	53	Wheat middlings
		(Mean: 68.8)	
3	D17	93	Control
3	D22	82	Vitamin B deficient
3	C11	64	Milk
3	C14	59	Steenbock's
		(Mean: 74.5)	
4	D6	54.5	Wheat middlings
4	C8	51.5	Control
		(Mean: 53)	
5	C13	57	Steenbock's
5	D21	56	Vitamin B deficient
		(Mean: 56.5)	
6	C1	43	Control
7	D10	47	Wheat middlings
7	D7	44	Wheat middlings
7	C9	41	Milk
		(Mean: 44)	
8	D14	36	Soy bean oil meal
9	C12	38	Steenbock's
10	C3	37.5	Vitamin G deficient
11	C4	39	Vitamin G deficient
12	D18	31.3	Control
14	C2	38	Control
42	C6	32	Control

heavy infestations than in light ones, but the difference of size of worms in light and heavy infestations was slight as compared to the variation between those of similar severity of infection in different dogs. Andrews (4) reports that, as the population of the sheep nematode *Cooperia curticei* increased, the egg production per female worm was found to decrease.

In *Hymenolepis fraterna* infections in rats and mice, Shorb (46) found that after the eighth day the larger worms were present in the hosts having fewer parasites and he suggests that, as the worms grow, crowding becomes a factor in retarding growth.

CONCLUSIONS

1. There is no direct relationship between the number of rat tapeworms harbored and the number of eggs eliminated daily by the rat host.

2. There is an inverse relationship between the number of worms harbored by a rat and the average number of eggs produced by each worm, a fact possibly attributable to crowding.

3. There is a tendency for tapeworms to be shorter when present in large numbers within a host than when present singly.

4. When large numbers of worms are harbored, some of the worms may fail to develop gravid proglottids and to produce eggs.

5. Spontaneous loss of the tapeworm *Hymenolepis diminuta* does not occur readily from the rat.

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[1] Conclusions for Parts I, II, and III will be found on pages 131, 139 and 144 respectively.

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A METHOD FOR ESTIMATING THE RED FOX POPULATION[1]

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Recognition of the importance of sustained yield in fur has stimulated the wildlife manager to develop methods of estimating the extent of this resource. Information has been obtained only by alert and continued observations, supplemented by seasonal survey and a well-planned census, or inventory.

In response to demands for increased knowledge of the widely distributed red fox (*Vulpes regalis*) and striped skunk (*Mephitis m. avia*) populations, Scott and Selko (1939) suggested the use of data obtained by a count of rearing dens on sample areas. This method was found satisfactory, but its use in determining red fox populations emphasized the desirability of a supplemental technique requiring less time, energy, and funds. An inventory technique is herein described to supplement or to replace the den count.

In the development of technique as used for the rearing den census, little consideration was given direct observation as a means of estimating populations. The den count includes the finding of the rearing dens and the interpretation of the detailed evidence presented by assignment of an average number of individuals to the den. The inventory herein described requires the finding and the interpretation of localized "sign," but the evidence is not brought to such definite focus as in the rearing den count. The evidence here considered is merely the "sign" created by recent, normal activity of red foxes within the home range. The interpretation, following Raunkiaer's system, is based on a percentage determination of the frequency of such activity on sample units drawn at random from the census area.

EVIDENCE OF OCCUPATION

Determining the presence of red foxes from the characteristic "sign" in the field demands some experience, a fact which cannot be over emphasized, but the requirements are not so exacting as to eliminate practice of the technique by qualified game managers.

The track made by foot pads is the most reliable and frequent form of evidence. The general shape of the red fox track is oval with the longer axis taking the direction of travel. There is some variation between the

fore and hind tracks, but on most tracking surfaces these differences are too obscure to be easily determined or are of such nature as not to affect seriously the identity of the track. In comparison with the front foot the hind foot is slightly narrower, the heel pad is not so wide and the two middle toes together with their claws usually appear to converge. Rarely are tracks of red foxes more than 2.8 inches long, and the writer has yet to find one that is wider than 75 per cent of the measured length. The toe pads are small, and the toes may be spread, particularly in the front foot and on soft surfaces. The bar-shaped heel pad leaves a rectangular print, straight-edged to the rear. The heel pad does not project forward between the outermost toes, and claw marks are usually present and sharper than those of the domestic dog. On such surfaces as mud and wet snow that are fine enough to hold the impression, the furry nature of the foot may be clearly observed. The track patterns of the different gaits are similar to those of the domestic dog except that the tracks of the walking or trotting fox are usually in almost perfect registry, in line and not obviously staggered.

Tracks are best seen on surfaces bare of vegetation. The best examples of these are ditch and gully bottoms, sand bars, roads and bare shoulders along streams, and stock trails. Topographic features and cover formations that provide gateways and avenues offer clews, especially concerning the probable line of travel. Cultivation of land for agricultural purposes also provides tracking opportunities, the "dead" furrows in plowed ground being especially used. On occasion soil disturbances like those produced by the pocket gopher are attractive to foxes.

The value of the scent has been emphasized by Stebler (1939). Frequently one familiar with fox scent will catch the odor before other "sign" has been detected. The scent posts commonly observed in dry tracking are small boulders more or less isolated and averaging about 12 inches in diameter. Boulders are probably located because they are abundant and wet urine may be seen on their surfaces at a distance. These and other scent posts may be found along stream beds, gully bottoms, and stock trails.

The scat, or dropping, indicates presence, but the identity of its originator is more difficult to determine than that of other "sign". The appearance and dimensions of the scat vary somewhat with the kind of food taken. For the most part the scats are largely made up of mammal remains. They are usually cylindrical, compact, sectioned, rounded, and frequently drawn out in a string or tail on the ends. One hundred scats from caged wild red foxes that had been fed small mammals and some domestic chickens were measured at their largest diameters. The average was 12.53 mm., the smallest 8.2 mm. and the largest 16.4 mm. Scats containing the remains of such foods as fruit and insects are usually larger in diameter. There is a coating of mucus over the scat until removed by insects or weather. The characteristic odor associated with the fresh scat remains for a few days. On occasions, as when much fruit is eaten, this odor is suppressed by the stronger odor of the food remains.

Scats are usually deposited in bare areas, hence, the search for tracks will carry the investigator over the most likely spots. Within these areas the fox will defecate almost anywhere, nevertheless, attention may well be given certain favored places. Slightly elevated and bare areas where the soil has been kicked up by livestock, prominences formed by erosion, and flat-topped rocks seem especially attractive. Cow and horse dung, leather straps, prey, and more or less isolated items, including sticks, small stones, and nuts, also appear to be attractive places for droppings.

Evidence of predation may assist in determining the presence of red foxes. In areas where food is abundant there may be food caches, which may or may not be buried. Weasels, shrews, and moles have been found with skulls crushed and uneaten. Apparently not so desirable as food, these prey species are killed and left uneaten, a habit that has likewise been noted in the domestic cat. Nests of cottontail rabbits and mice are regularly excavated, and mole runways are ripped open, the dead mole often being found nearby. The bases of rotted stumps that have been dug into by foxes apparently seeking mice may be noted.   -

Though the "signs" of the presence of red foxes have been discussed at some length, it does not follow that all of them must be noted to prove the presence of foxes in any one locality. Unquestionable identification of tracks or scent should be the basis for the determinations. Other evidence, although good, should serve more in the capacity of assisting investigations.

These "sign" readings then provide essentially for an appraisal of the occupied home ranges of fox families. If the readings are made in early summer prior to August, families may be identified from the presence of the tracks of adults and young. Later in the year, as when the inspections here presented were made, it is not practicable to segregate tracks of young from those of adults.

APPLICATION

In order to obtain data on an adequate scale over large areas, a sampling method was necessary. Sections (640 acres) were considered desirable units. Samples were drawn at random on a countywide scope, 576 sections in area. In this study, samples totaling five per cent of the entire area appeared adequate; under other circumstances, however, greater accuracy may be obtained in larger acreages, smaller sample units, or both.

It is suggested that the data obtained will be more accurate if the area from which the samples are to be drawn is classified. Scott and Selko (1939) demonstrated a correlation between the density of rearing dens of red foxes and striped skunks, and land slopes that have a predominant gradient of 5 to 10 per cent or more. Support for this correlation was also found in the occupational-frequency determinations. This information is suggested as a basis for classifying census areas whenever

applicable. Thus, with the use of a topographic map or from familiarity
with the land one can divide an area to be studied into two classes; one
including lands that have a slope predominantly of 5 to 10 per cent or
more and the remaining lands. A 5 per cent sample may be drawn from
the area with slopes of less than 5 to 10 per cent and a 10 per cent sample
for the steeper slopes. This minimizes the time consumed for the less
productive area and tends to concentrate efforts on the more productive
area.

The work is planned in advance so as to save time in the field. Each
sample area is covered, starting at the most vulnerable point—the place
most likely to yield evidence. An up-to-date map will be a help in deter-
mining where this point will be. As drainage systems are especially pro-
ductive when the stream levels are below normal, the approach to the
sample area should begin where the water course leaves the strip. Obser-
vations begun at the more promising localities will reveal evidence of fox
occupation within a few minutes. Surveys should be delayed for at least
two days after a heavy rain so as to permit accumulation of "sign". As the
most readable and most reliable evidence is collected after a rain it is
advisable to wait for such conditions. The method has not been applied to
red fox populations in areas also inhabited by gray foxes (*Urocyon cinereo-
argenteus*), but it would seem that with additional care the "sign" of the
two species could be segregated and results obtained.

Although different localities may require some variation in time of
inspection, the period September 15 to November 15 seems most satisfac-
tory. Within this period, in order to make comparisons, counts on the
different sample areas should be made as near concurrently as possible.

The method here described was attempted only during the fall as a
pre-winter count. With allowances, however, it may possibly be used at
other times of the year.

INTERPRETATION

The data gathered from the sample area should be given a frequency
value. This is done by finding what the percentage of the number of
samples on which "signs" of presence is found is of the total number of
samples studied. For example, an inspection of 50 sample sections showed
evidence of fox occupation on 10; this indicated an occupational fre-
quency, or index, of 20 per cent.

A survey was made of the red foxes in Boone County during Novem-
ber of two consecutive years. The survey revealed a frequency index of
40 per cent in 1938 and 12 per cent in 1939. As the rearing den count was
also made in the same county in these two years, the trends in population
as indicated by the two methods may be compared. The count of rearing
dens in 1938 was 6 and in 1939, 2. Thus when the occupational-frequency
method showed a drop in population from 40 to 12 per cent, the rearing-
den count showed a drop from 39 to 13 per cent. No limiting factor that
produced a perceptible change in populations during the period between

the den count and the inventory was recorded or reported during the two years studied.

An inventory technique that seems to show trends in population as accurately as is indicated by these percental determinations, combined with yield records, should provide administrators with basic information on which to adjust harvest operations over large areas. Skill in practice should tend to increase the value of the data obtained by this technique.

The futility of applying exact figures to constantly changing populations is recognized; it appears forgivable, however, to suggest a possible translation of occupational frequency into numbers of individuals. The data from the November frequency inventories in Boone County indicate that a maximum occupational frequency, 100 per cent, would represent an average population of about 1.6 foxes to the section. This figure was obtained from the available data as follows: In 1938 the red fox population on the 576 sections in Boone County was 351, as determined from the den count; in the same year the frequency index was 40 per cent. The population at maximum frequency, 100 per cent, was then reckoned to be 878 or about 1.52 to the section. In 1939 the den count indicated a population of 117, and the frequency index was 12 per cent. The population at maximum frequency was 975 to 576 sections or about 1.69 foxes to the section. The average number of foxes to the section at maximum frequency would be approximately 1.6. The average fox population to the section may then be obtained by multiplying 1.6 by the percental determination of occupational frequency.

SUMMARY

The inventory technique herein described is based on a percental determination of the frequency of red fox occupied sample units (640-acre sections) in a series drawn at random from a census area. The frequency determinations appeared responsive to annual population changes.

In practice the technique requires a minimum of time, energy, and funds, and is suited to large-scale application. Administrators should find this technique efficient and reasonably reliable in providing information on which to base recommendations for harvest of the red fox fur surplus. Thus, a seed stock for continued production will be insured; foxes will be available throughout the year for those who find recreation in coursing them with hounds; and the surplus may be taken for profit.

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THE EFFECT OF ASPECT OF SLOPE ON CLIMATIC FACTORS[1]

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In a given geographic location modifications of climatic factors induced by aspect of slope are known to exist although few quantitative determinations have been made of the magnitude of these modifications either in general or in specific instances. Previous microclimatic studies (Geiger (3), Aikman (1), Eisele (2), Potzger (7)) have dealt chiefly with the climate of plant habitats, the modifications in which may be attributable to variations in vegetative cover alone or to variations in both topography and vegetative cover.

As a result of quantitative studies of microclimate, it has been suggested (Geiger (3), Aikman (1)) that the determination of climatic factors affecting plant growth should be made for each habitat in a given geographic location provided the modification in factors is sufficient to affect significantly the development of the plants.

The present study was undertaken to determine the magnitude of differences in climatic factors, independent of any measurable effect of modifications in plant cover, on four aspects of slope within a radius of one-fourth mile at the Hillculture Field Station near Floris in southern Iowa. The initiation of adaptation studies of a number of species and varieties of plants introduced into the area for the first time made it necessary to measure and evaluate, in this particular location, the degree of modification of climatic factors resulting from topographic differences alone. The purpose of this paper is to present data showing the effect of aspect of slope on the individual climatic factors and on the integrations of climatic factors which seem to have the greatest effect on the establishment and growth of plants.

DESCRIPTION OF THE STATIONS

The four stations are located on north, east, south, and west slopes within a radius of one-fourth mile on the farm. All of the stations are on slopes of about 20 per cent, and are of approximately the same elevation with the exception of that on the north slope which is about 60 feet higher than the others.

Of the four stations, those on the east and west slopes have practically no protection by trees and shrubs for a distance of at least 200 feet on the north and south and for a distance of 300 feet on the east and west. The south slope is unprotected for only 125 feet on the south but for at least

200 feet on the west and for more than 300 feet on the east. The north slope is unprotected on the north for 125 feet, on the west for 75 feet and on the east for 300 feet. The upper slopes of the four sites are devoid of woody plant cover and afford about the same degree of protection.

Each station was equipped with the following instruments: United States Weather Bureau standard maximum-minimum thermometers; Julien P. Friez hygrothermograph and soil-air thermograph (soil temperature depth, 2 inches); Robinson cup anemometer (cups at 2-foot height); three porous cup atmometers (cups at 6-inch height); and an accurate non-recording rain gauge. In addition to these instruments, the station on the south slope had a soil thermograph at the 8-inch depth and a Julien P. Friez dual-traverse survey type recording rain and snow gauge. The instruments were housed in shelters of modified Weather
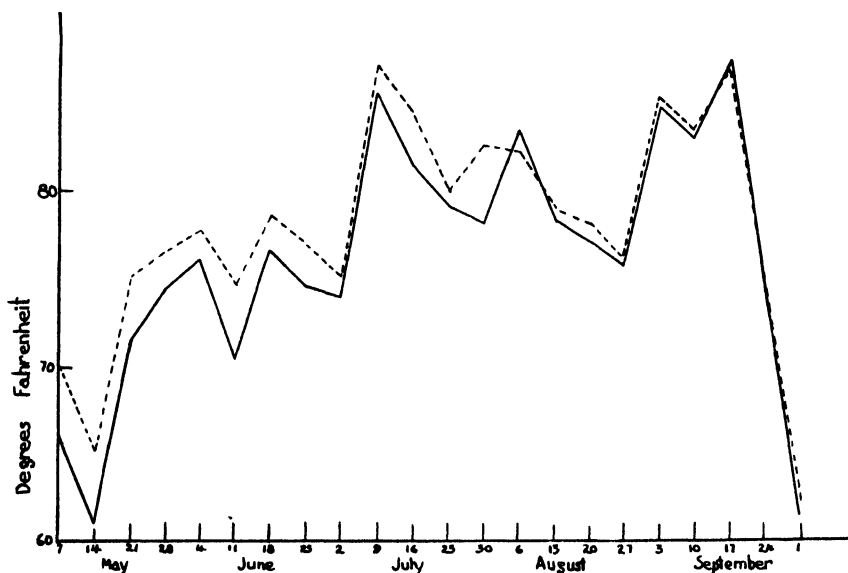
Fig. 1. Comparison of average diurnal air temperature of east slope (entire line) and of west slope (broken line). 1939.

Bureau type, painted green. The instruments at each station were located in a plot 15 by 20 feet, which was kept mowed at a height of two inches.

COMPARISON OF CLIMATIC FACTORS

In the selection of climatic factor data to be presented in this paper, an attempt has been made, from a study of the literature and from the response of introduced plants tested near the stations on the east and west slopes, to evaluate the several factors so that only those are presented that appear to have a definite effect on the establishment and growth of plants.

For most of the factors there seems to be a greater difference between

the stations on the east and west slopes than between any other two stations. The added protection of the other two slopes may to some extent account for this fact.

AVERAGE TEMPERATURE

The average temperature at each station was determined by obtaining the mean of readings at two-hour intervals from thermograph charts. Average temperature differences of apparently small magnitude materially affect plant growth since the difference in effective heat units between two habitats is the product of the daily average temperature difference and the number of days of the record. These relatively small variations in average temperature are especially important in their effect on plant development at or near average plant zero and in the higher temperature range, near the optimum for most plants, where growth responses are magnified by temperature increases.

In figure 1 a comparison of average diurnal air temperatures for the east and west slopes shows that for the first part of the season the average difference was between 2 and 3 degrees. The average difference between the night temperature of the two slopes (not plotted) was slightly less than this. The average difference between the temperature for both day and night was more than two degrees for the two stations, which would seem to have some appreciable effect on growth of plants since the difference in mean summer air temperature between southeastern Minnesota and northern Missouri is only 5 degrees (Kincer (4)).

Average air temperature for July 1939 varied 3 degrees between the south and west slopes and almost as much between the east and west slopes (table 1). This difference equals the average July temperature difference between the position of the farm in southern Iowa and a location south of the Missouri River in Central Missouri, a distance of 150 miles (Kincer (4)).

TABLE 1. *Monthly averages of air temperature, maximum air temperature, and soil temperature (2-inch depth) in degrees Fahrenheit on the north, east, south, and west slopes, July, 1939*

Locations of stations	Average air temperature	Average maximum air temperature	Average soil temperature 2-inch depth
North slope	78.0	94.0	78.8
East slope	76.8	93.6	81.2
South slope	75.4	93.5	80.2
West slope	78.5	. . .	85.8

Average soil temperature at the 2-inch depth, shown in table 1, differed as much as 7 degrees between the slopes. Such wide variations in the upper soil temperature have a marked effect on the temperature and hence on the metabolism of the plants growing on the slopes.

Although the differences in maximum and minimum temperature between slopes reached 6 and 8 degrees, respectively, these differences were unusual rather than the rule. Most of the differences were 1 to 3 degrees, the maximum temperature differences being greater in the middle of the growing season and the minimum differences greater at the beginning and toward the end of the growing season. Table 1 shows that the average of the maximum temperature readings on three of the slopes for July 19 varied only one-half degree Fahrenheit. The free circulation of air at the stations probably accounted for the small difference in average maximum temperature on the slopes. The average of the three stations for July, 1939, was almost 7 degrees higher than the 40 year average (Reed (5)).

In contrast, maximum soil temperature differences were great. On July 7, 1939, the temperature of the soil at the 2-inch depth reached 118 degrees Fahrenheit on the west slope compared to 98 degrees Fahrenheit on the east slope. Previous tests showed that the soil temperature just below the surface was several degrees higher. The injury caused by such excessive heat at or near the surface of the soil seems to be an important factor in explaining the failure of plants to become established on partially bare sites.

ALTERNATE FREEZING AND THAWING

There was considerable variation among the stations in the number and duration of periods showing alternate freezing and thawing throughout the fall, winter, and spring of 1938-39 as computed from the weekly temperature charts and checked with the maximum-minimum thermometers. For the south slope, the number of these periods was 115 as compared to 105 for the west slope. Incomplete records of the other two slopes showed that the north slope was more favorable than the west slope in this regard, probably because of increased protection, and that the south slope was about half way between the east and west slopes. Slight differences in extent of direct frost injury to transplanted material and of injury from heaving might be demonstrated for the four sites.

LENGTH OF GROWING SEASON

The average length of growing season for the area, based on 40 years record, is about 165 days (Reed (5), (6)). The four stations differed widely in frost-free days in 1939, as determined by minimum thermometers and checked with the weekly thermograph charts. The number of frost-free days for north, east, south, and west slopes were, respectively, 176, 162, 162, and 201. In 1939 there was a 30 day difference in the first frost date in the fall. No data are available for early spring, 1938, but the first frost date for 1938 for all stations falls on October 24, indicating that there was little variation in frost-free days among the stations for 1938.

Differences among the slopes in length of growing season seem to vary widely from year to year depending on the direction of the wind and the

degree of protection. This length of season is an important factor in its effect on the growth of introduced plants. The data for 1939 indicate that the variation in frost date may be as wide between adjacent slopes of different aspect as between two locations at least 200 miles north and south.

<div align="center">RATE OF EVAPORATION</div>

Although the relationship between rate of evaporation and transpiration from plants is somewhat indefinite since it has been shown that the correlation between these two determinations is not very high, the comparative dryness of the atmosphere as measured by porous cup atmometers is another factor of some use in the evaluation of plant habitats.
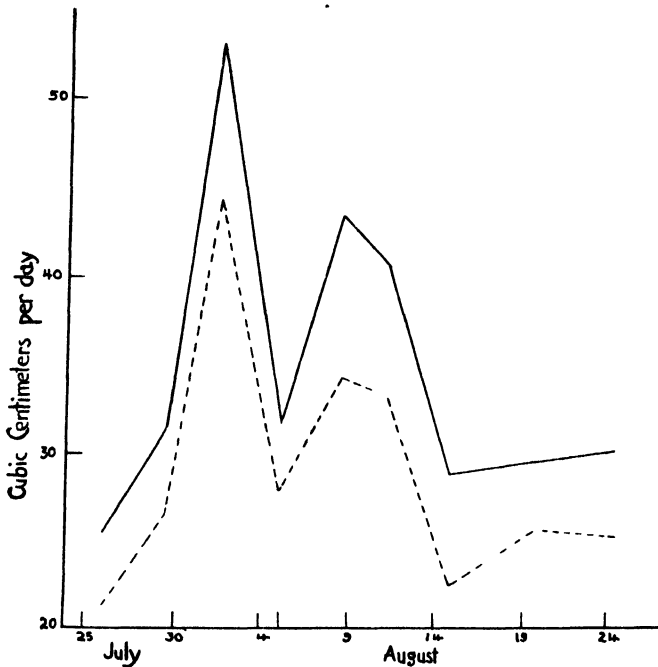
Fig. 2. Comparison of rate of evaporation from atmometers in cubic centimeters per day on 20 per cent west slope, (entire line) and on 20 per cent east slope (broken line) within a distance of 250 yards. 1938.

Figure 2 shows the rate of evaporation in cubic centimeters per day from porous atmometers on the east and west slopes. Evaporation averaged about 6 cubic centimeters per day higher for the west slope than for the east slope during the period represented by the graph. Several shorter periods during the two seasons showed evaporation to be greater for the west slope by more than 20 per cent; and in one instance 40 per cent. Granted that the saturation deficit of the turbulent zone of the upper atmosphere was approximately the same over the two sites, the gradient

between the surface air and this area would be much greater for the west slope than for the east slope.

Average relative humidity, determined from hygrograph chart readings at 2-hour intervals, for these two sites for the period July 25 to August 24, 1938, were 74 and 78 per cent for the east and west slopes, respectively, and the average air temperature readings were 76 and 75 degrees Fahrenheit. The average wind velocity (figure 3) for this period was more than 60 per cent greater on the west than on the east slope. The mean
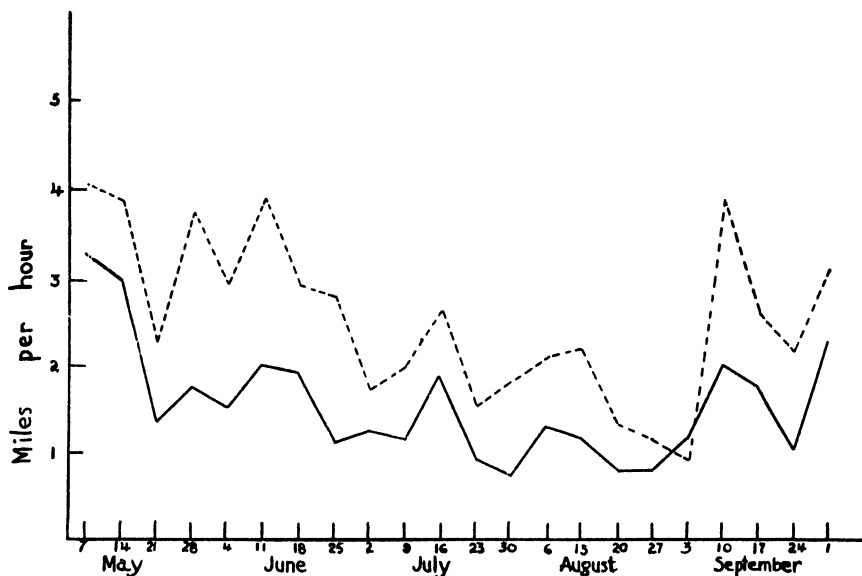
Fig. 3. Average daily wind velocity in miles per hour on east slope (entire line) and on west slope (broken line) anemometer cups at two-foot height. 1939.

annual air temperature was about 2½ degrees Fahrenheit higher on the west slope than on the east slope.

Curves plotted from values representing the product of average temperature, wind velocity, and saturation deficit of the air show a correspondence to the rate of evaporation curves for the two slopes and indicate that this combination of factors, which also showed a significant correlation with transpiration rate, is of greater magnitude on the west slope than on the east slope.

SUMMARY

1. Quantitative differences induced by variation in aspect of slope, within a given geographic location in southern Iowa, are presented for climatic factors selected on the basis of their effect on plant growth. The north and south slopes were somewhat better protected by woody vegetation than the east and west slopes.
2. Under the conditions of the experiment there seemed to be the greatest contrast in all factors between the east and west slopes.

3. The west slope had the highest average air and soil temperature, the highest maximum soil temperature at 2 inches, the longest frost-free season, the highest evaporation, the greatest wind velocity, and, next to the north slope, had the smallest number of alternate freezing and thawing periods.
4. The west slope was the most xeric, with the other slopes ranking in the following order: south, east, and north.
5. On the basis of heat injury the slopes would rank, in order: west (greatest danger of injury), south, east, and north.
6. On the basis of cold injury alone the slopes would rank as follows: east (greatest danger of injury), south, north, and west.

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THE DISTRIBUTION OF IOWA TOADS[1]

REEVE M. BAILEY AND MARIAN K. BAILEY

In the course of an investigation of the amphibians and reptiles of Iowa it has become apparent that no less than four toads occur, although only the American toad *(Bufo a. americanus)* has heretofore been reported. The locality records now at hand, based chiefly upon collections of breeding specimens, indicate that one or more species probably occur in each county in the state, and that no species is found throughout the state. We are grateful to A. Cooke Anderson, Max E. Davis, Dr. George C. Decker, Lewis T. Graham, Dr. Harold Gunderson and other staff members and students of the Iowa State College who have participated in the collection of specimens.

Although identification is at times difficult because of the variability of differentiating characters, we have as yet noted no evidence of intergradation or hybridization of the forms. *Bufo woodhousii fowleri* and *B. w. woodhousii*, which are believed to intergrade further south, are well separated geographically in Iowa (fig. 2), and the geographic source of material furnishes a simple method for separation of the subspecies. The following key, based entirely on Iowa material, should facilitate proper identification.

Key to the Toads (Bufo) of Iowa

1a.—Interorbital crests heavy, strongly converging anteriorly and uniting to form a pronounced boss between anterior margins of eyes. Dorsal blotches larger than parotids, conspicuously light margined, including 7 (usually at least 10) or more enlarged warts. Smaller metatarsal tubercle provided with a definite, though dull, cutting edge. Belly immaculate. Throat pouch of breeding male sausage shaped, emerging well back on throat, and, when not distended, covered by a flap of normally colored skin. *Western two tiers of Iowa counties.*
> GREAT PLAINS TOAD *(Bufo cognatus)*

1b.—Interorbital crests often less well developed, parallel or moderately converging but not uniting to form a boss. Dorsal blotches much smaller than parotids, without light margins, each involving 1 to 5 (rarely more) enlarged warts. Smaller metatarsal tubercle bluntly conical or knob-like, without a cutting edge. Belly immaculate to heavily mottled or spotted. Throat pouch of breeding male subhemispherical, dark colored, involving all of loose skin on throat.

2a.—Breast and belly more or less heavily mottled or marbled with dark. Parotid glands broader and more closely approximated, parotid width 1.5 to 2.0 (usually 1.8 or less) in its length and 0.8 to 1.6 (usually less than 1.3) in least interparotid distance. Warts on body stronger and fewer, those on tibia especially well developed. Cranial crests usually moderately converging anteriorly; postorbital crests separated from parotids. Mid-dorsal light stripe narrower and contrasting little with dorsal coloration, sometimes indistinct. First (distal) subarticular tubercle of longest toe almost invariably divided (96 per cent); second subarticular tubercle most often divided (64 per cent). Song a high-pitched, protracted, musical trill, normally of more than 10 seconds duration. *Throughout Iowa except western edge of state.*   AMERICAN TOAD *(Bufo americanus americanus)*

2b.—Underparts immaculate or with a single, median, dark spot on breast (occasionally with several dark flecks on breast). Parotid glands narrower and less closely approximated, parotid width 1.6 to 2.6 (usually 1.8 or more) in its length and 1.3 to 2.6 (usually 1.5 or more) in least interparotid distance. Warts on body smaller and more numerous, those on tibia less well developed. Cranial crests typically almost parallel; postorbital crests normally contacting parotids. Mid-dorsal light stripe broader and contrasting sharply with dorsal coloration (except in pallid specimens). First (distal) subarticular tubercle of longest toe often single (34 per cent in *fowleri*, 54 per cent in *woodhousii*); second subarticular tubercle never divided. Song a rather low-pitched, nasal "waa-a-a-a", of less than 5 seconds duration.

3a.—Dorsal dark spots involving 3 or 4 (occasionally 2 or 5, rarely 1 or more than 5) warts; total wart count in six spots[2] 14 to 31 (15 or more in 96 per cent), mean 19.8. Parotid width 1.3 to 1.9 (1.8 or less in 92 per cent), mean 1.7, in interparotid distance. Postorbital ridge in contact with tympanum in adults. Size smaller, largest male examined 66 mm. in snout to vent length, largest female 65 mm. (doubtless attaining a somewhat greater size). *Southeastern Iowa.*   FOWLER'S TOAD *(Bufo woodhousii fowleri)*

3b.—Dorsal dark spots involving 1 or 2 (occasionally 3, rarely 4 or 5) warts; total wart count in six spots[2] 6 to 18 (13 or less in 92 per cent), mean 10.1. Parotid width 1.7 to 2.6

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[2] The six spots counted include the two lying between anterior ends of parotids, two between posterior ends of parotids, and two lying near mid-dorsal line in middle of back.

(1.9 or more in 88 per cent), mean 2.1, in interparotid distance. Postorbital ridge rarely in contact with tympanum. Size larger, largest male examined 89 mm. in snout to vent length, largest female 103.5 mm. *Western two tiers of Iowa counties.*   ROCKY MOUNTAIN TOAD *(Bufo woodhousii woodhousii)*

AMERICAN TOAD

Bufo americanus americanus Holbrook

(Figure 1 and Plate I, figures A and B)

The American toad is abundantly represented, to the exclusion of other toads, throughout most of Iowa (figs. 1 and 2). It has been taken in
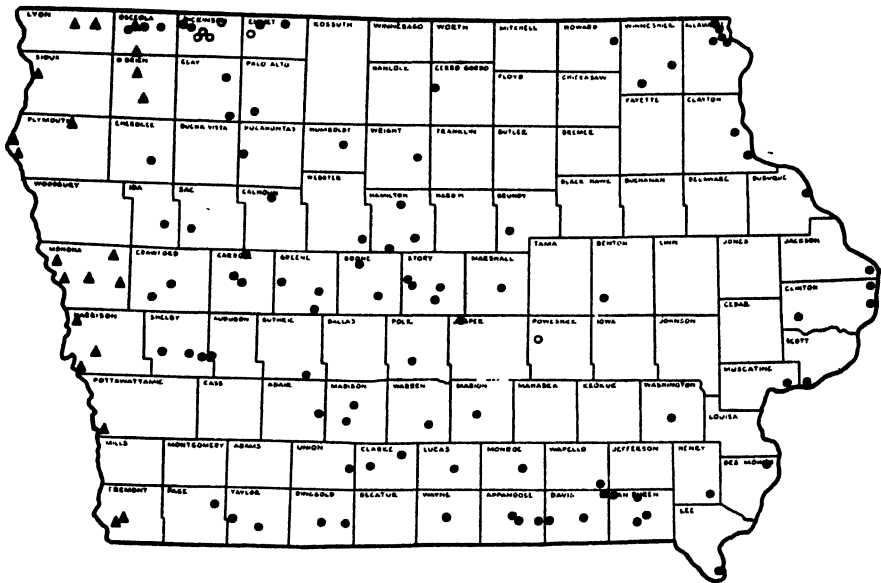
Fig. 1. Map of Iowa showing locality records for *Bufo a. americanus* (circles) and *Bufo cognatus* (triangles). Literature reports for *americanus* appear as open circles.

company with *fowleri* in Lacey-Keosauqua State Park, Van Buren County and with *woodhousii* near Ida Grove, Ida County, at Denison, Crawford County, and along Keg Creek, 5.7 miles west of Harlan, Shelby County. At Minden, Pottawattamie County, we heard this species and *woodhousii* singing on May 11, 1940, but only *woodhousii* was collected. Although not thus far collected with *cognatus* the ranges overlap slightly in Osceola and perhaps other western counties and they doubtless occur together. However, the Iowa range of *americanus* is to a remarkable extent complementary with those of *woodhousii* and, especially, *cognatus*, a circumstance not improbably resulting from interspecific competition.

Athough toads abound in the Missouri and Bix Sioux River valleys

we have never taken *americanus* there, and it is believed that the species does not range west to the western boundary of Iowa. To the south the western range limit in Kansas, as determined by Smith (1934: 439), is continuous with that in Iowa (fig. 1). If the species occurs in Nebraska, which seems doubtful, it is presumably confined to the extreme south-eastern corner of that state. We are informed by Dr. William H. Over that he has no records of this species from South Dakota; the toads reported by him (1923) as *B. americanus* are *B. w. woodhousii*. The western range to the north extends at least to the Red River valley where we
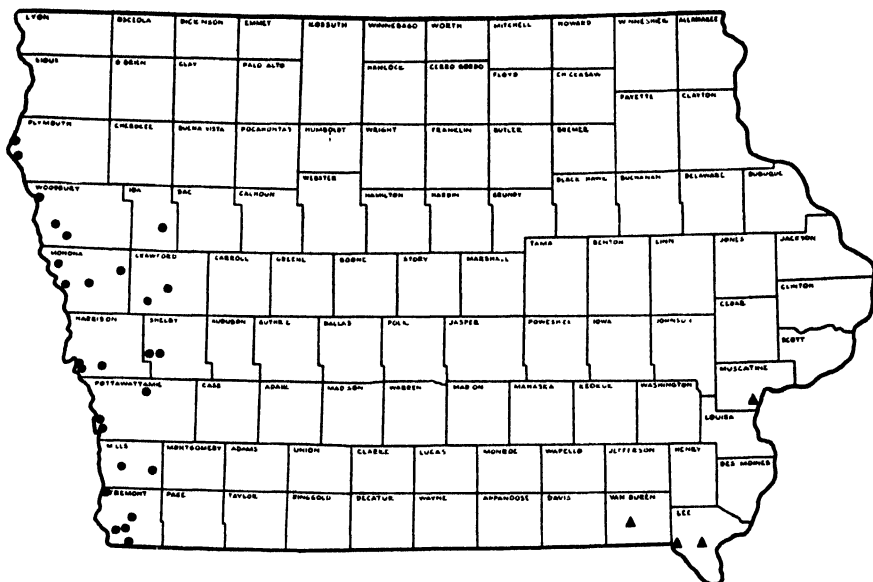
Fig. 2. Map of Iowa showing locality records for *Bufo woodhousii fowleri* (triangles) and *Bufo w. woodhousii* (circles).

have taken *americanus* in company with *cognatus,* along U. S. Highway 75, 5 miles south of Wolverton, Wilkin County, Minnesota. The species is said to range west to Alberta (Stejneger and Barbour, 1939:31).

Iowa records are as follows (all specimens of this and the other forms are in the Iowa State College collection unless otherwise designated. If more than one specimen was collected the number follows the locality in parentheses. Individuals found dead on roadways are designated DOR and are not preserved): ADAIR Co., 10.5 miles east of Greenfield. ALLAMAKEE Co., 1 mi. S Lansing (DOR); 2 mi. SW New Albin; 3 mi. W Lansing; 4.5 mi. NW Lansing (not preserved); Church (DOR). APPANOOSE Co., 4.3 mi. N Centerville (2); 1.8 mi. N Centerville (DOR); 9.5 mi. E Centerville (DOR); 2.8 mi. E Centerville; 4.6 mi. N Centerville (DOR). AUDUBON Co., Kimballton (3). BENTON Co., 4 mi. SSW Keystone (DOR). BOONE Co., 1 mi. E Pilot Mound; Ledges State Park (6). CALHOUN Co., west shore of North Twin Lake (DOR). CARROLL Co., 2.5 mi. SE Carroll; 1 mi. W Carroll; 6 mi. NNW Lidderdale. CERRO GORDO Co., 1 mi. S Ventura (2). CHEROKEE Co., 5 mi. S Cherokee (2). CLARKE Co., 9.5 mi. W Osceola (2); 8.4 mi. NE Osceola (22). CLAY Co., (Ruthven, 1910: 203, 205, in the Museum of Zoology, University of Michigan); 5 mi. SE Webb; 2.5 mi. NE Dickens. CLAYTON Co., Garnavillo;

Guttenberg. CLINTON Co., 12 mi. N Clinton; 4 mi. N Clinton; 2 mi. E Wheatland (DOR). CRAWFORD Co., 0.5 mi. W Dow City; 0.5 mi. W Denison (4). DAVIS Co., 2.5 mi. SE Eldon (DOR); 3.7 mi. W West Grove; 2.4 mi. W West Grove; 4 mi. E Bloomfield (2). DES MOINES Co., 3 mi. N Kingston (DOR). DICKINSON Co., Iowa Lakeside Laboratory; N shore West Lake Okoboji; Upper Gar Lake; Little Sioux River; East Lake Okoboji; Lower Gar Lake (all reported by Blanchard, 1923: 21, MZUM); Silver Lake, 2 mi. SSW Lake Park; 7.6 mi. W Spirit Lake (DOR). DUBUQUE Co., 2.5 mi. N Dubuque. EMMETT Co., 1 mi. W Estherville (DOR), (Scott, 1938:530); 2 mi. NE Dolliver (not pres.); 6 mi. NE Estherville. GREENE Co., 4 mi. E Jefferson; 3.5 mi. N Scranton; 10 mi. S Jefferson. GRUNDY Co., 7 mi. W Grundy Center. GUTHRIE Co 1 mi. S Menlo. HAMIL-TON Co., 3 mi. S Jewell (3); 6 mi. E Webster City (9); 4.4 mi. S Stanhope (DOR). HENRY Co., 2.5 mi. N. Lowell. HOWARD Co., 2 mi. N Cresco (not pres.). HUMBOLDT Co., 3.5 mi. E Arnold (2). IDA Co., 1.5 mi. E Ida Grove (8); Ida Grove. JACKSON Co., 2 mi. NW Sabula. JASPER Co., 9.5 mi. SW Melbourne. LEE Co., 3.5 mi. N Keokuk; 5 mi. N Keokuk. LUCAS Co., 1 mi. E Chariton (not pres.). MADISON Co., 5.5 mi. N Winterset (DOR); 4.5 mi. SW Winterset (not pres.); 6 mi. WSW Winterset (8). MARION Co., 2 mi. W Knoxville (2). MARSHALL Co., 9 mi. E State Center (DOR); 7 5 mi. E State Center. MONROE Co., 3 mi. E Albia (3); 5.9 mi. E Albia (DOR). MUSCATINE Co., 2 mi. NE Fairport (2). OSCEOLA Co., 1 mi. N Sibley; 2.5 mi. NE Allendorf (DOR); 1.5 mi. NE Ocheyedan. PAGE Co., 6 mi. NE Clarinda. PALO ALTO Co., (Ruthven, 1910: 203, 205, MZUM); Rush Lake, 8 mi. W Mallard. POCAHONTAS Co., Clear Lake,.11 mi. W Pocahontas. POLK Co., Des Moines (4). POWESHIEK Co., Grinnell (Ruthven, 1912: 207, MZUM). RINGGOLD Co., 2 mi. NW Mt. Ayr (DOR). SAC Co., Odebolt. SCOTT Co., 1.3 mi. W Buffalo. SHELBY Co., 3.6 mi. SE Jacksonville; 5.7 mi. W Harlan; 5.9 mi. E Harlan (2). STORY Co., Skunk River, Ames (2); 5 mi. S Nevada; 2.8 mi. E Nevada; 5 mi. NW Ames. TAYLOR Co., 0.5 mi. SE New Market; 2 mi. NE Bedford; 0.5 mi. E Bedford (4). UNION Co., 9 mi. E Afton (40). VAN BUREN Co., 1 mi. SW Keosauqua (2); 1 mi. NW Selma (DOR); 2.2 mi. S Birmingham (DOR); 1.3 mi. SE Mt. Zion (DOR); 5 mi. ENE Keosauqua. WAPELLO Co., Eldon (DOR). WARREN Co., 5.1 mi. N Liberty Center. WASHINGTON Co., 2 mi. W Washington (DOR). WAYNE Co., 1 mi. W Corydon (DOR). WEBSTER Co., 4 mi. WSW Stratford (DOR). WINNESHIEK Co., Ft. Atkinson (tadpoles); 8.5 mi. SE Decorah (2). WRIGHT Co., 1.5 mi. W Rowan (DOR).

GREAT PLAINS TOAD

Bufo cognatus Say

(Figure 1 and Plate I, figure C)

The occurrence of *cognatus* in southwestern Minnesota (Breckenridge, 1938: 47) presaged its discovery in Iowa. It has been taken in Osceola and O'Brien counties in northwestern Iowa; all other records are from those counties bordering the western edge of the state. It occurs abundantly in company with *woodhousii* in the Missouri River valley, where these species replace *americanus*.

Iowa localities are as follows: FREMONT Co., 7 mi. SW Sidney (2); 6 mi. N Hamburg (DOR). HARRISON Co., 3 mi. SE River Sioux (DOR); 5 mi. N Missouri Valley (DOR); 3 mi. S California (2). LYON Co., 1 mi. E Rock Rapids (DOR); 7.6 mi. E Rock Rapids (DOR). MONONA Co., 1 mi. W Mapleton (DOR); Badger Lake, 3.5 mi.. W Whiting (2); 1 mi. N Turin (28); 3.5 mi. W Onawa (3); 5 mi. SW Ute. O'BRIEN Co., 2.8 mi. N Sanborn (2); 4.1 mi. S Primghar (DOR). OSCEOLA Co., 2.5 mi. SSW Melvin (DOR); 1.5 mi. N Allendorf. PLYMOUTH Co., 4 mi. S Westfield (6); 4.3 mi. SW Akron (DOR); 6.8 mi. N LeMars (DOR); 2 mi. SSW Akron (7). POTTAWATTAMIE Co., 4.5 mi. SSE Council Bluffs (DOR). SIOUX Co., 2 mi. NE Hudson, S. Dak. (DOR).

FOWLER'S TOAD

Bufo woodhousii fowleri Hinckley

(Figure 2 and Plate I, figures E and F)

The relationship of *fowleri* and *woodhousii* has been discussed in detail by Smith (1934: 456-457), and we are in accord with his conclusion that the forms are conspecific.

The few Iowa localities for the eastern subspecies, *fowleri*, are confined to the southeastern part of the state. The only singing individuals were taken July 21, 1939, in Lee County. The song impressed us as less raucous and slightly more musical than that of *woodhousii*, but we doubt our ability to separate the subspecies consistently by sound alone.

Iowa localities are as follows: LEE Co., 6 mi. E Donnellson (58); 3 mi. ESE Farmington. MUSCATINE Co., 4.5 mi. SW Muscatine (5). VAN BUREN Co., 1 mi. SW Keosauqua (25).

ROCKY MOUNTAIN TOAD

Bufo woodhousii woodhousii Girard

(Figure 2 and Plate I, figure D)

Bufo w. woodhousii, like *cognatus*, is a western toad which reaches its eastern limit in western Iowa. Our northernmost locality is in Plymouth County from which it ranges south into Missouri. We have taken specimens from 3 to 9 miles north of Carrollton, Carroll County, in that state. Although not extending as far north in Iowa as *cognatus*, *woodhousii* appears to occur somewhat further east as indicated in figures 1 and 2.

On the night of May 11, 1940, we established an east-west transect from Kimballton, Audubon County, to Portsmouth, Shelby County. At Keg Creek, 5.7 miles west of Harlan, both *americanus* and *woodhousii* were taken. East of that locality only *americanus* was collected or heard, and west of it only *woodhousii*.

Iowa localities are as follows: CRAWFORD Co., 0.5 mi. W Denison (2); 3 mi. SW Dow City. FREMONT Co., 6 mi. N Hamburg; 1 mi. NW Bartlett (2); 2.8 mi. E Sidney (DOR); 7 mi. SW Sidney (6); Hamburg; 2 mi. W Riverton. HARRISON Co., 2.4 mi. N Missouri Valley; 3 mi. S California (6); 5 mi. W California. IDA Co., 1.5 mi. E Ida Grove (3). MILLS Co., 1.3 mi. W Emerson (DOR); 3 mi. SE Glenwood (2). MONONA Co., Blue Lake, 3.7 mi. W Onawa (DOR); Badger Lake, 3.5 mi. W Whiting; 5.5 mi W Whiting (DOR); 7 mi. SE Mapleton; 1 mi. N Turin (10); 4 mi. W Onawa (9). PLYMOUTH Co., 4 mi. S Westfield (2); 2 mi. SSW Akron (2). POTTAWATTAMIE Co., 4.5 mi. SSE Council Bluffs (DOR); 2.5 mi. N Council Bluffs; Minden (9). SHELBY Co., Portsmouth; Keg Creek, 5.7 mi. W Harlan (5). WOODBURY Co., Sioux City (4); Holly Springs (DOR); 8.4 mi. NW Holly Springs.

SUMMARY

Three species of toads occur in Iowa, one of which is represented by two subspecies. Of these *Bufo cognatus* and *B. w. woodhousii* are western forms which, in Iowa, attain their eastern range limits in the western two

tiers of counties. *Bufo a. americanus* and *B. woodhousii fowleri* are primarily eastern in distribution and reach their western range limits in Iowa; *fowleri* has penetrated into the southeastern portion of the state whereas *americanus* has invaded all of Iowa except the western edge. The distribution of *americanus* in the state is roughly complementary with those of *cognatus* and *woodhousii*, a fact perhaps to be attributed to competition.

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PLATE I. THE TOADS OF IOWA

Fig. A.   *Bufo a. americanus:* a breeding male 75 mm. in snout to vent length, from 6 miles east of Webster City, Hamilton County.

Fig. B.   *Bufo a. americanus:* ventral view, a breeding male 58 mm. in snout to vent length, from the same locality.

Fig. C.   *Bufo cognatus:* an adult male 71.5 mm. in snout to vent length, from near Akron, Plymouth County.

Fig. D.   *Bufo w. woodhousii:* an adult female 73.5 mm. in snout to vent length, from 3 miles south of California, Harrison County.

Fig. E.   *Bufo woodhousii fowleri:* an adult female, 57 mm. in snout to vent length, from near Muscatine, Muscatine County.

Fig. F.   *Bufo woodhousii fowleri:* ventral view, an adult female 66 mm. in snout to vent length, from the same locality.

Photographs by Max E. Davis

PLATE I. THE TOADS OF IOWA
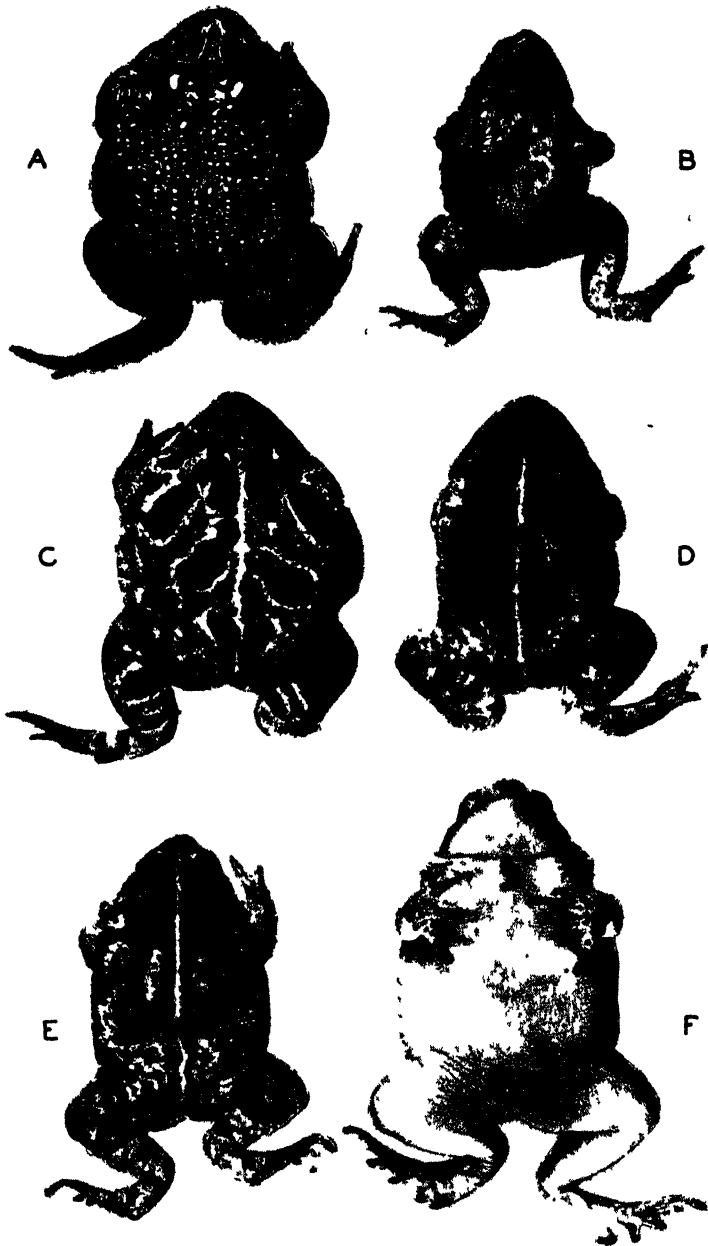
THE VARIATIONS IN GALIUM TRIFLORUM AND GALIUM BOREALE

Philip J. Leyendecker, Jr.

From the Department of Botany, Iowa State College

Galium triflorum, a variable species common to the New England and midwestern states was described by Michaux in 1803.

During a recent study of the genus in Iowa this species was found to segregate into three distinct morphological forms. Further examination of additional material from other states revealed that these three forms tend to be geographically distinct. The writer is, therefore, proposing three forms of *G. triflorum.* The material examined for this paper is in the Herbarium of the Missouri Botanical Garden, and the Iowa State College Herbarium.

Galium triflorum Michaux, Fl. Bor. Am. 1: 80. 1803.

Perennial, stems diffuse or reclining, 12-40 cm. long, hispid, retrorsely barbed or glabrous on the angles; leaves 6 in a whorl, elliptical to elliptical-lanceolate, tapering at the base, 2-7 cm. long, cuspidate pointed, 1-nerved, veiny, margins and midribs scabrous; flowers terminal and axillary; peduncles mostly 3-flowered at the extremity; corolla 4-parted, greenish to greenish-white, 3-4 mm. across, lobes acute to acuminate; fruit 2-2.5 mm. across, beset with long, hispid, uncinate hairs.

Galium triflorum Michx. forma typicum. (*Galium triflorum* Michx. Fl. Bor. Am. 1: 80. 1803.)

Angles of the stems retrorsely barbed. Type locality: "In umbrosis Canadae sylvis." This form ranges across the United States from New Brunswick south to Florida, New Mexico, California, Oregon, Washington, and Alaska.

Representative material: NEWFOUNDLAND: Sept. 7, 1896; A. C. Waghorne 35; NEW HAMPSHIRE: Crawford Notch, July 3, 1898, J. M. Greenman 1065; NEW YORK: Sullivan Co., June, 1873, H. Eggert; MASSACHUSETTS: Lincoln, Aug. 28, 1898, J. M. Greenman 2905; PENNSYLVANIA: Montgomery Co., July 5, 1899, A. Mac Elwee 781; NEW JERSEY: Milberg, July 10, 1880, J. Schrink; DISTRICT OF COLUMBIA: Anacostia, July 19, 1893, F. L. Boettcher 194; WEST VIRGINIA: Huntington, July 8, 1937, F. A. Gilbert, 594; VIRGINIA: Fauguier Co., June 30, 1935, H. A. Allard 735; NORTH CAROLINA: Swain Co., Aug., 1891, H. C. Beardslee; GEORGIA: De Kalle Co., July 23, 1897, H. Eggert; FLORIDA: Aspalaga, July, 1843, Rugel; ALABAMA: Cullman Co., Sept. 26, 1898, H. Eggert; TENNESSEE: Reelfoot Lake, June, 1932, G. E. Moore B34; KENTUCKY: Harlon Co., 1893, T. H. Kearney, Jr. 232; OHIO: Cincinnati, July 6, 1890, C. G. Lloyd; INDIANA: Blackford Co., Aug. 6, 1905, C. C. Deam 150; ILLINOIS: La Salle Co., June-Sept., 1921, F. H. Thone 128; MINNESOTA:

Clearwater Co., July 9, 1929, M. L. Grant 2708; IOWA: Des Moines, Oct. 18, 1895, G. W. Carver; MISSOURI: Jasper Co., June 28, 1909, E. J. Palmer 2375; ARKANSAS: Little Rock, June, 1835, Geo. Engelmann 674; TEXAS: Dallas, June 27, 1907, J. Reverchon 2607; OKLAHOMA: Pawhuska, Aug. 9, 1913, G. W. Stevens 1892; KANSAS: Manhattan, July 24, 1893, A. S. Hitchcock; NEBRASKA: Fort Pierre, June, 1853, F. V. Hayden; MONTANA: MacDougal Peak, July 31, 1908, Mrs. J. Clements; COLORADO: Greenhorn Mts., Sept. 14, 1875, T. S. Brandegee 2840; IDAHO: Priest Range Exp. Sta., July, 1923, C. C. Epling 6187; NEVADA: Mountain City, Aug. 9, 1912, Aven Nelson and J. F. Macbride 2187; CALIFORNIA: Lake Co., July 25, 1902, A. A. Heller 5982; OREGON: Wallowa Co., June 29, 1897, E. P. Sheldon 8432; WASHINGTON: Seattle, July 9, 1937, J. W. Thompson 7285; BRITISH COLUMBIA: Vancouver Isl., June 30, 1893, John Macoun; ALASKA: Kodiak Isl., July 28, 1938, E. H. Looff and H. B. Looff 493.

Galium triflorum Michx. forma *hispidum* Leyendecker n. form.

Angles of the stems beset with straight hairs. Otherwise as the species. (Angulis caulum setosis. Aliter similis speciei.)

Type: Ledges State Park, Boone Co., Iowa, July 25, 1903. L. H. Pammel, R. E. Buchanan and C. M. King 3934. (Iowa State College Herbarium).

This form ranges from New Brunswick south to West Virginia, Missouri, New Mexico, California, Washington, Oregon, and Alaska.

Representative material: MAINE: Piscataquis Co., June 24, 1895, M. L. Fernald 223; VERMONT: Smugglers Notch, July 5, 1897, J. M. Greenman 67; MASSACHUSETTS: Purgatory Swamp, June 27, 1897, J. M. Greenman 67; PENNSYLVANIA: Bucks Co., July 21, 1895, collector unknown; VIRGINIA: Luroy, Aug. 18, 1901, E. S. Steele 14; WEST VIRGINIA: Pocahontas Co., June 16, 1896, W. M. Pollock; MICHIGAN: Mackinac, Sept., 1896, H. H. Babcock; WISCONSIN: Door Co., July 10, 1918, M. T. Greenman 23; IOWA: Clinton, Sept. 4, 1896, L. H. Pammel 245; MISSOURI: Marion Co., July 26, 1915, John Doves 1444; NEBRASKA: Nuckolls Co., July, 1896, G. G. Hedgcock; SOUTH DAKOTA: Deadwood, July 9, 1913, P. A. Rydberg 38; NORTH DAKOTA: Abercrombie, June 25, 1912, H. F. Bergman 1772; MONTANA: Spring Hill, Aug. 27, 1905, J. W. Blankinship 239; WYOMING: Centennial Valley, Aug. 17, 1895, A. Nelson 1693; COLORADO: Ruxton Brook, July 23, 1901, F. E. and E. S. Clements 250; NEW MEXICO: Pecos River, Aug. 20, 1898, G. E. Coghill 175; CALIFORNIA: Fresno Co., June 25-July 15, 1900, H. M. Hall and H. P. Chandler 349; UTAH: San Juan Co., July 2, 1932, B. Maguire and J. D. Redd 2123; IDAHO: Custer Co., July 19, 1916, J. F. Macbride and E. B. Payson 3331; OREGON: Jackson Co., May 31, 1892, E. W. Hammond 183; WASHINGTON: Bengen, June 20-July 28, 1903, W. N. Suksdorf 2844; BRITISH COLUMBIA: Field, June 28, 1906, S. Brown 349; ALASKA: Prince of Wales Isl., Sept. 12, 1915, Mr. and Mrs. E. P. Walker 1005.

This form does not extend as far southeast as the form *typicum*.

Galium triflorum Michx. forma *glabrum* Leyendecker n. form.

Angles of the stems glabrous. Otherwise as the species. (Angulis caulum glabris. Aliter similis speciei.)

Type: Webb City, Jasper Co., Missouri, July 23, 1910, B. F. Bush 6029. (Herbarium of the Missouri Botanical Garden).

This form ranges from Quebec south to Virginia, Arkansas, Texas, Arizona, Iowa, Minnesota, and again appears on the Pacific coast in California, Washington and British Columbia.

Representative material: NEWFOUNDLAND: Aug. 13, 1894, B. L. Robinson and H. Schrenk 32; QUEBEC: Aug. 18, 1927, J. Rosseau 26947; MAINE: Augusta, Aug. 25, 1887, E. C. Smith; VERMONT: Brandon, June 25, 1923, D. L. Dutton; NEW YORK: Geneva, June 21, 1882, E. L. Sturtevant: MASSACHUSETTS: Hampden Co., Sept. 15, 1913, F. C. Seymour 90; PENNSYLVANIA: Sellersville, June, 1881, C. D. Fritz; WEST VIRGINIA: Pocahontas Co., June 16, 1896, W. M. Pollock; KENTUCKY: Barren Co., July 20, 1900, S. F. Price; ONTARIO: July 24, 1893, J. Fowler; IOWA: Lee Co., July 21, 1931, J. L. Fults 1506; MISSOURI: Marion Co., Aug. 13, 1913, John Davis; ARKANSAS: Mt. Mena, Oct. 1, 1898, Trelease; TEXAS: Buzzard Springs, 1875, J. Reverchon 386; ARIZONA: White Mts., Aug. 6-15, 1903, D. Griffiths 5342; IDAHO: Payette Lake, July 24, 1899, M. E. Jones 6374; WASHINGTON: Mt. Angeles, Aug. 2, 1930, J. W. Thompson 5485.

It may be noted that this form is somewhat more limited in distribution than either of the forms *typicum* or *hispidum*.

Galium boreale L. Sp. Pl. 108. 1753.

In the course of this study some four hundred sheets of *G. boreale* L. were examined. The specimens fell into three previously described groups, based on fruit characters. Professor Fernald[1] found the same to be true of material in the Gray Herbarium and the Herbarium of the New England Botanical Club. Most of the material examined by the writer was from the Herbarium of the Missouri Botanical Garden, and is reported on primarily because it further extends the known ranges of the varieties.

Galium boreale L. var. *typicum*. Material examined from Manitoba, Sask., Minn., Wisc., Ind., Ill., Ia., Nebr., S. Dak., N. Mex., Colo., Ut., Id., Wyo., Mont., Ore., Wash., Brit. Col., Alb., and Alas.

Galium boreale L. var. *intermedium* DC. Material examined from N. Y., Vt., Conn., Mass., N. J., Penn., Del., W. Vir., Ohio, Ind., Ill., Mich., Wisc., Minn., Brit. Col., Ont., Alb., Mo., Ia., Nebr., S. Sak., N. Dak., Tex., N. Mex., Colo., Ut., Id., Wyo., Mont., Calif., Ore., Wash., Vancouver Isl., and Alas.

Galium boreale L. var. *hyssopifolium* (Hoff.) DC. Material examined from N. Y., Mass., Wisc., Mich., Minn., Ill., Mo., Ia., and Wash.

Considerable difficulty was encountered in drawing a clear line between the varieties *typicum* and *intermedium*. In many cases the specimen in question could be referred to either variety. The author, because of this difficulty, is inclined to regard the three varieties as forms at most. Since the original descriptions were drawn from European material, he hesitates to reduce these three varieties to forms without thorough study of additional European specimens.

For aid in the preparation of the above paper the writer is deeply grateful to Dr. G. J. Goodman, Curator of the Iowa State College Herbarium. He also wishes to thank Dr. J. M. Greenman for the loan of specimens in the Herbarium of the Missouri Botanical Garden.

[1] Fernald, M. L., Rhodora, 30:106-107. 1928.

MOLLUSKS IN THE VICINITY OF AMES, IOWA

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From the Department of Zoology and Entomology, Iowa State College

The following list of snails and mussels was compiled from collections made by the author in and around Ames in the fall of 1940 at the times specified: (1) Campus of Iowa State College along Clear Creek and Squaw Creek, Sept. 30th; (2) Skunk River near Ames above the mouth of Squaw Creek, with Carl Riggs, Oct. 5th; (3) Central campus of Iowa State College, Oct. 9th; (4) Skunk River near Ames below the mouth of Squaw Creek, with Eric Isakson, Oct. 12th; (5) Squaw Creek, five miles north of Ames, near Gilbert, with Dr. E. R. Becker, Oct. 17th; (6) Clear Creek above the campus, Oct. 22nd; (7) Squaw Creek at the bridge crossing Riverside Drive, Nov. 2nd. The study represents a portion of the research of the author in the Graduate School of Iowa State College. In addition to the above mentioned who aided in the collecting, acknowledgement is due Dr. C. J. Drake, head of the Department of Zoology and Entomology for facilities, and to Dr. E. R. Becker for further aid and encouragement. Also all specimens have been left with Dr. Becker.

The records contribute to the extension westward of the range of many of these species. The Ames area, as a transition region between the timbered valleys of eastern Iowa and the great plains of Nebraska, supports a correspondingly depauperate fauna as the species "taper off" westwardly. The records may be of later significance in interpreting biologic fluctuations in this area, particularly as such are affected by the innovations of man. As the second largest phylum in the animal kingdom, the Mollusca, itself economically unimportant, is of considerable importance as a source of food for economically-important groups as fishes and game birds. Certain members of the *Fossaria* group in other regions harbor the economically-destructive fluke-parasites of sheep and cattle. Some aquatic mollusks are also efficient scavengers, aiding in the recovery of streams from pollution.

The species are numbered in systematic order under their respective categories. Localities in the list below are designated by arabic numerals* in parentheses, which correspond to the numbers of the localities given above. To avoid .confusion the number of specimens taken is given not as a numeral but as a written word.

Phylum MOLLUSCA (Linnaeus) Cuvier
　Class GASTROPODA CUVIER
　　Subclass STREPTONEURA Spengel
　　　Order CTENOBRANCHIATA Schweigger
　　　　Suborder PLATYPODA Lamarck
　　　　　Family VALVATIDAE Gray
　　　　　　Genus *Valvata* Müller

1. *Valvata tricarinata* (Say) — (7), two.

Family AMNICOLIDAE (Tryon) Gill
Genus *Amnicola* Gould and Haldeman

2. *Amnicola limosa* (Say) — (4), two; (2), three; (7), four.

Genus *Cincinnatia* Pilsbry

3. *Cincinnatia cincinnatiensis* (Anthony) — (2), two; (7), five.

Family POMATIOPSIDAE Stimpson
Genus *Pomatiopsis* Tryon

4. *Pomatiopsis lapidaria* (Say) — (7), four.

Family PLEUROCERIDAE Fisher
Genus *Pleurocera* Rafinesque

5. *Pleurocera acuta* Raf. — (4), eleven; (2), three.

　Subclass EUTHYNEURA Spengel
　　Order PULMONATA Cuvier
　　　Suborder STYLOMMATOPHORA A. Schmidt
　　　　Family POLYGYRIDAE Pilsbry
　　　　　Genus *Mesodon* Rafinesque

6. *Mesodon clausus* (Say) — (1), four. The largest land snail found. Dr. Becker has specimens of larger species, *Triodopsis multilineata* (Say) and *Mesodon zaletus* (Binney) that he collected from timbered areas, now cleared, near Boone, Iowa.

Genus *Stenotrema* Rafinesque

7. *Stenotrema monodon* (Rackett) — (4), three; (2), eight; (7), seven.
-　Some doubt the advisability of separating this species from *fraterna*. While intermediate specimens may occur in this territory, the two species are separable. Size and habitat differences (*monodon* is smaller and lives in lowland pastures along streams) are more constant features than the open or covered condition of the umbilicus. At locality (7) the two species were separable, though living together.

8. *Stenotrema fraternum* (Say) — (1), three; (7), two; (6), one.

Family ZONITIDAE Pfeiffer
Genus *Retinella* (Shuttleworth) Fischer

9. *Retinella electrina* (Gould)—Formerly *Vitrea hammonis* (Ström). (1), one; (6), five; (2), one; (7), four.

10. *Retinella indentata* (Say)—(5), one; (6), four; (7), one.

Genus *Hawaiia* Gude

11. *Hawaiia minuscula* (Binney)—(6), three; (5), three; (2), one; (7), eight.

Genus *Zonitoides* Lehmann

12. *Zonitoides arboreus* (Say)—(5), ten; (6), sixty-two; (4), thirty-three; (2), sixteen; (7), three.

Family ENDODONTIDAE Pilsbry
Genus *Anguispira* Morse

13. *Anguispira alternata* (Say)—The tiger snail. (7), one juvenile. Dr. Becker has mature specimens from Boone, Iowa.

Genus *Discus* Fitzinger

14. *Discus cronkhitei anthonyi* (Pilsbry)—(7), six.

Genus *Helicodiscus* Morse

15. *Helicodiscus parallelus* (Say)—(5), four; (6), two; (2), one; (7), six.

Genus *Punctum* Morse

16. *Punctum pygmaeum* (Draparnaud)—The pygmy snail. (7), two.

Family PUPILLIDAE Turton
Genus *Gastrocopta* Wollaston

17. *Gastrocopta armifera armifera* (Say)—(5), one; (6), two; (7), nine.

18. *Gastrocopta armifera similis* (Sterki)—(4), one.

19. *Gastrocopta armifera affinis* (Sterki)—(6), six; (7), five.

20. *Gastrocopta contracta* (Say)—(6), ten; (2), two; (7), six.

21. *Gastrocopta corticaria* (Say)—(5), three.

Genus *Pupoides* Pfeiffer

22. *Pupoides marginatus* (Say)—(6), two; (7), three.

Family STROBILOPSIDAE*
Genus *Strobilops* Pilsbry

23. *Strobilops labyrinthica* (Say) — (1), two.

Family VALLONIIDAE*
Genus *Vallonia* Risso

24. *Vallonia costata* (Müller) — (7), two.

Family COCHLICOPIDAE*
Genus *Cochlicopa* (Ferussac) Risso

25. *Cochlicopa lubrica* (Müller) — (6), two; (7), one.

Family SUCCINEIDAE Albers
Genus *Succinea* Draparnaud

26. *Succinea avara* (Say) — (6), three; (4), two; (2), two; (7), one.

Family LIMACIDAE Lamarck
Genus *Deroceras* Rafinesque

27. *Deroceras agreste* (Linnaeus) —Formerly *Agriolimax agrestis* (L.)
The European slug. (6), three; (3), one; (2), two; (7), two.

Family ELLOBIIDAE H. and A. Adams
Genus *Carychium* Müller

28. *Carychium exiguum* (Say) — (6), one.

Suborder BASOMMATOPHORA A. Schmidt
Family LYMNAEIDAE Broderip
Genus *Stagnicola* Leach

29. *Stagnicola caperata* (Say) — (7), two.
Genus *Fossaria* Westerlund

30. *Fossaria obrussa* (Say) — (6), nine; (2), one; (7), sixteen.

Family PLANORBIDAE H. and A. Adams
Genus *Helisoma* Swainson

31. *Helisoma antrosum* (Conrad) —Formerly *Planorbis bicarinatus* Say.
The bicarinate ram's horn snail.— (6), one; (4), five; (2), six; (7),
twenty-three.

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* Author's name at present not available.

Genus *Planorbula* Haldeman (*Segmentina* Fleming)

32. *Planorbula crassilabris* (Walker)—(2), one; (7), three.

Genus *Gyraulus* Charpentier

33. *Gyraulus parvus* (Say)—(6), one; (7), two.

Family PHYSIDAE Dall

Genus *Physa* Draparnaud

34. *Physa sayii* Tappan—(2), two; (4), two; (7), seven.

35. *Physa gyrina* Say—(2), one; (4), two; (7), eight. Dr. Becker has many specimens from Gilbert, Iowa.

36. *Physa integra* Haldeman—(5), one; (6), four; (4), two; (7), ten.

Class LAMELLIBRANCHIATA Blainville (PELECYPODA Goldfuss)

Order PRIONODESMACEA Dall

Family UNIONIDAE (d'Orbigny) Ortmann

Genus *Fusconaia* Simpson

37. *Fusconaia flava* (Rafinesque)—Formerly *Quadrula rubiginosa* (Lea). Creek-form of the pig-toe mussel. (5), two; (4), four.

Genus *Amblema* Rafinesque

38. *Amblema plicata costata* (Rafinesque)—Formerly the *Quadrula undulata* of authors. The three-ridge. (5), five pairs of valves.

Genus *Lasmigona* Rafinesque

39. *Lasmigona complanata* (Barnes)—Formerly *Symphynota complanata* (Barnes). White heel-splitter. (4), one.

Genus *Anodonta* Lamarck

40. *Anodonta grandis* Say—Floater. (4), one.

Genus *Strophitus* Rafinesque

41. *Strophitus rugosus pavonius* (Lea)—Creek-form of the Squaw Foot Mussel. (4), two.

Genus *Carunculina* Simpson

42. *Carunculina parva* (Barnes)—Formerly *Lampsilis parva* (Barnes). The Lilliput shell. (4), two paired valves and one unpaired valve; (2), one.

Genus *Ligumia* Swainson

43. *Ligumia recta latissima* (Rafinesque)—Formerly *Lampsilis recta* (Lam.) The river-form of the Black Sand Shell. (5), two.

Genus *Lampsilis* Rafinesque

44. *Lampsilis siliquoidea* Barnes—Formerly *Lampsilis luteola* (Lam.)
The river-form of the Lake Pepin mucket. (5), three male valves,
one female; (4), three male valves.

Order TELODESMACEA Dall
Superfamily CYRENACEA Tryon
Family SPHAERIIDAE Dall
Genus *Sphaerium* Scopoli

45. *Sphaerium striatinum* (Lamarck)—The striate seed-shell. (5), four
unmatched valves; (4), sixteen unmatched; (2), ten unmatched;
(7), five pairs, with young shells within adult valves.
46. *Sphaerium solidulum* (Prime)—The solid seed-shell. (4), twenty-
two unmatched valves.

Genus *Musculium* Link

47. *Musculium truncatum* (Linsley)—The truncate seed-shell. (4), one
valve.

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THE EFFECT OF TREATED FATS ON VITAMIN A POTENCY. II[1]

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Previous work on this subject has been reported by Harrelson, Nelson, Lowe, Dyme and Nelson (1). It is not necessary to review the literature concerning this investigation since the above article refers to essential papers bearing on the subject at hand. Annual reports (2) of the progress of this work have mentioned that the fundamental experiments which led to these studies were done by Fridericia (3) who first observed that hydrogenated whale oil, when mixed with fat containing vitamin A, caused destruction of this vitamin. The investigation herein reported was concerned primarily with the effect of treated fats upon vitamin A potency rather than upon vitamin A itself.

EXPERIMENTAL AND DISCUSSION

All of the experiments were performed on rats. With the exception of the animals employed for toxicity experiments, they weighed between 45 and 60 gms. when placed on the various rations. The animals used for toxicity investigations were mature animals. All of the rats were obtained from the stock colony maintained by the Chemistry Department at Iowa State College. The animals employed for the experimental work were placed in screen-bottom cages. Six rats, three males and three females, were placed in each lot. They were weighed weekly and cared for daily. The basal ration used in the tests, with the exception of the experiments on toxicity, consisted of the following: casein 200 gms., salts 50 gms., yeast 100 gms., and dextrin 450 gms. The animals for toxicity experiments received the growing ration which has been used successfully in this laboratory for many years and which is composed for the most part of naturally occurring food materials. Casein was prepared by washing the commercial product with a 0.15 per cent solution of acetic acid until it was free of vitamin A. The salt mixture was similar to that employed by McCollum and Davis (4) with the exception that it contained small amounts of copper and manganese salts and a small amount of potassium iodide. The yeast consisted of a dried product from Standard Brands, Inc. Dextrin was prepared by treating starch with a dilute solution of citric acid and subjecting the mixture to 15 pounds steam pressure for three hours in an autoclave. The butter employed in the experiments was obtained from the Dairy Industry Department at Iowa State College. Butterfat was prepared by heating the butter on a steam plate to the

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melting point, after which the fat was promptly decanted from the curd, the salts, and the water.

Previous work (1) had shown that certain fats when heated in a one-eighth inch layer and subsequently mixed with a vitamin-A-bearing oil destroyed this vitamin. This work was continued and additional fats were studied. The results are given in table 1. Four rations, similar to those employed by Fridericia (3), were used in this study: ration I consisted of 800 gms. of the basal ration and 200 gms. of butterfat; ration II consisted of 900 gms. of the basal ration plus 100 gms. of butterfat; ration III was composed of 800 gms. of the basal ration plus 100 gms. of butterfat and 100 gms. of the fat undergoing investigation; and ration IV consisted of 800 gms. of the basal ration, 100 gms. of butterfat, and 100 gms. of the corresponding heated fat investigated. The heat treatment consisted of subjecting a one-eighth inch layer of the fat to a temperature of 102°C. to 105°C. for 24 hours. The butterfat and the fat tested were mixed at temperatures slightly above the melting points of the two fats. Growth of the animals was normal on rations I and II.

With the exception of one sample of coconut oil (Exp. N) all of the heated fats inactivated butterfat. Heated Mazola, heated cod liver oil, and heated cottonseed oil inactivated cod liver oil (Exps. L, S, and W); heated open kettle lard, heated Laurel-leaf lard, and heated butterfat failed to do so (Exps. H, I, and T). Heated soybean oil inactivated haliver oil (Exp. 2). The animals receiving the heated fats which failed to inactivate cod liver oil did not grow so well as the animals on the corresponding unheated fats. This would seem to indicate a partial destruction of vitamin A activity. The experiments indicated that the vitamin A activity of cod liver oil was more resistant to the destructive action of heated fats than was that of butterfat. Furthermore, they show that various fats responded differently to the action of heat. It will be observed that all of the fats which, upon being heated, inactivated cod liver oil had iodine numbers of 100 or more, a value much higher than was possessed by those fats such as lard and butterfat, which upon heating failed to inactivate cod liver oil. The degree of unsaturation of the fat was not the only factor involved in its response to heat, since all but one of the animals receiving the heated cod liver oil (Exp. S) remained alive during the experimental period although they exhibited subnormal growth. On the other hand, those animals died which received heated Mazola (Exp. L) and heated cottonseed oil (Exp. W), both of which were more saturated than cod liver oil. Five of the animals fed heated cod liver oil were living at the end of 19 weeks while the animals fed heated Mazola and heated cottonseed oil were all dead by the eighth and sixteenth weeks respectively. The animals on heated cod liver oil had an average weight of 96 gms. at 19 weeks as compared with an average weight of 219 gms. for the controls receiving the unheated cod liver oil (Exp. S). Further evidence that rancid oils destroyed vitamin A is presented by Experiment Z; peanut oil, rancidified at room temperature, inactived butterfat.

Cod liver oil, forming one per cent of the diet, supplied sufficient vitamin A for a rather slow rate of growth of rats, provided other fats in the diet had not been previously heated. However, if heated lard formed ten per cent of the diet, such a concentration of cod liver oil failed to produce growth, and the animals died. Diets which contained ten per cent of heated lard were capable of supporting growth when greater concentrations of cod liver oil (2.5 per cent, 5.0 per cent, 7.5 per cent and 10 per cent) were employed. However, growth of the animals fed the heated lard at each level of cod liver oil was slower than the growth exhibited by the animals fed the unheated lard at corresponding levels of cod liver oil. The partial or complete inactivation of cod liver oil, brought about by heated lard, is dependent to a considerable extent upon oxidation products of cod liver oil. Using heated-lard diets, it was found that an increase in the amount of cod liver oil beyond a certain concentration (five per cent) caused a decrease in growth. Apparently at the higher concentrations of cod liver oil the increased amount of decomposition products, which were formed in the cod liver oil, more than offset the beneficial effect of the extra vitamin supplied by this oil.

Experiments were conducted upon products of fat hydrolysis to determine which components of the fat were so changed by heat as to bring about destruction of vitamin A activity. Stearic, palmitic, and oleic acids, as well as glycerol, were tested before and after heating in the usual manner (a one-eighth inch layer heated at 102° C. to 105° C. for 24 hours). Heated oleic acid was the only one of these four substances which inactivated butterfat. This demonstrated that it was the unsaturated portion of a fat molecule which responded to heat as determined by its effect upon vitamin A activity. This was in agreement with the observation that the more unsaturated fats show a greater response to the action of heat.

Experiments were made to determine the minimum amount of heated lard which would inactivate butterfat when the latter comprised ten per cent of the diet. Seven and one-half per cent of heated open kettle lard inactivated the butterfat, whereas five per cent of the heated lard failed to do so. Experiments were also performed to ascertain the effect of the thickness of fat layer upon the response of the fat to heat; butterfat was employed as the source of vitamin A. Olive oil and Laurel-leaf lard were heated in layers of one-eighth, one-fourth and three-eighths inches thick. The controls received unheated fats. The effect of heat upon a fat is in roughly inverse proportion to the depth of the fat layer. The rats receiving the fats heated in the two thinner layers died; whereas the animals fed the fats heated in a three-eights inch layer survived on the olive oil but succumbed on the Laurel-leaf lard.

The reports of Anderegg and Nelson (5), Nelson, Jones, Adams, and Anderegg (6), and Marcus (7) upon the destructive action of finely divided solids on the vitamin A activity of cod liver oil suggested a

TABLE 1. *Effect of fats heated in a one-eighth inch layer (102°C.–105°C. for 24 hours) on vitamin A potency of butterfat, cod liver oil and haliver oil*

Exp.	Basic fat material	Trade name, description or source	Growth[c]		Xerophthalmia[d]		Vitamin A source[b]
			Unheated	Heated	Unheated	Heated	
A.	Olive oil	Pompeian	+	—	0	6	B.F.
B.	Lard	Laurel-leaf	+	—	0	1	B.F.
C.	Lard	Commercial[a]	+	—	0	6	B.F.
D.	Lard	open kettle (Wilson)	+	—	0	2	B.F.
E.	Lard	An. Husb. (I.S.C.)	+	—	0	5	B.F.
F.	Lard	An. Husb. (I.S.C.)	+	—	0	3	B.F.
G.	Lard	An. Husb. (I.S.C.)	+	—	0	5	B.F.
H.	Lard	open kettle (Wilson)	+	+	0	0	C.L.O.
L.	Lard	Laurel-leaf	+	+	0	0	C.L.O.
J.	Corn oil	Mazola	+	—	0	0	B.F.
K.	Corn oil	Mazola	+	—	0	1	B.F.[e]
L.	Corn oil	Mazola	+	—	0	0	C.L.O.
M.	Coconut oil	McKesson and Robbins	+	—	0	3	B.F.
N.	Coconut oil	McKesson and Robbins	+	+	0	0	B.F.
O.	Coconut oil	McKesson and Robbins	+	—	0	2	B.F.
P.	Coconut oil	McKesson and Robbins	+	—	0	4	B.F.
Q.	Cottonseed oil	Crisco	+	—	0	5	B.F.
R.	Cod liver oil	Squibb	+	—	0	6	B.F.
S.	Cod liver oil	Squibb	+	—	0	0	C.L.O.

T.	Butterfat	College	+	+	0	0	C.L.O.
U.	Butterfat	College	+	−	0	6	B.F.
V.	Cottonseed oil	Wesson	+	−	0	0	B.F.
W.	Cottonseed oil	Wesson	+	−	0	3	C.L.O.
X.	Palm oil	Wilkens-Anderson	+	−	0	5	B.F.
Y.	Soybean oil	Wilkens-Anderson	+	−	0	0	B.F.
Z.	Peanut oil (rancid)	Wilkens-Anderson	−	−	5	1	B.F.
1.	Peanut oil	Wilkens-Anderson	+	−	0	2	B.F.
2.	Soybean oil	Wilkens-Anderson	+	−	0	4	H.O.[f]
3.	Hydrogenated lard	Clix	+	−	0	6	B.F.
4.	Cottonseed oil	Crisco	+	−	0	0	B.F.
5.	Drip lard	French Oil Machinery Co., Piqua, Ohio	+	−	0	5	B.F.

[a] Commercial refers to purchase on the market.

[b] B.F. refers to butterfat; C.L.O. to cod liver oil; and H.O., to haliver oil.

[c] + signifies that body weight increased steadily and − signifies that loss of weight occurred after different lengths of time and that usually the animals died.

[d] Figures under xerophthalmia designate the numbers of animals showing symptoms of the disease; six animals were employed in each lot so that the remaining animals died without evidence of the disease.

[e] The bottles containing the rations in this experiment were kept in a refrigerator at 1°C. and only enough of the ration was taken out each day to feed the rats.

[f] In this experiment, 2 gms. of haliver oil, 25 gms. of soybean oil, and 623 gms. of dextrin were employed instead of the usual amounts.

similar study upon butterfat. Such a study was conducted to ascertain to what extent, if any, destruction of vitamin A activity in heated-fat diets was brought about by ingredients other than the fats. Fuller's earth and Norit A in 25 per cent concentrations were added to butterfat. These mixtures were then used as 10 per cent of the ration. Both the butterfat containing the Norit A, and that containing the fuller's earth, as well as an untreated sample of butterfat, were stored for specified periods in a refrigerator (1° C.) prior to use. The basal ration fed the animals consisted of 550 gms. of dextrin, 200 gms. of casein, 100 gms. of yeast, and 50 gms. of salts. The negative control lot received no butterfat but was given 100 extra gms. of dextrin.

Norit A and fuller's earth had very little effect upon the vitamin A activity of butterfat when they were mixed with this fat, the mixture stored in a refrigerator, and finally stirred with the basal ration and fed to the rats. The rations containing the mixtures which had been stored for eight months prior to use produced slower growth than those containing the mixtures stored for shorter periods; this effect was more pronounced with Norit A than with fuller's earth. Whereas the animals receiving 75 gms. of butterfat that had been stored for eight months possessed an average weight of 220 gms. at the end of the twelfth week, the rats receiving the butterfat-Norit A mixture and butterfat-fuller's earth mixture, also stored for eight months, weighed 155 and 192 gms. respectively at the end of this same period.

A study was made to ascertain whether the heated fats, when mixed with the vitamin-A-containing fat and fed apart from the basal mixture, would destroy vitamin A activity. The effects of heated Laurel-leaf lard on butterfat and heated cottonseed oil on cod liver oil were studied. Control groups received corresponding unheated fats simultaneously. The fats were mixed with an equal weight of the vitamin A source and stored in a refrigerator at 1° C. Enough of the fat solution was fed daily to equal 20 per cent of the food consumption. Heated Laurel-leaf lard destroyed vitamin A activity, but the action was slower than that observed when the mixed fats were incorporated in the basal mixture and kept at room temperature (table 1, Exp. B). The rats fed the heated lard and butterfat in the ration were all dead by the sixth week but none of the animals fed the solution of fats apart from the ration died before the eleventh week; all of the animals of this latter group were dead by the seventeenth week. Three of these animals exhibited xerophthalmia. Heated cottonseed oil inactivated cod liver oil only very slightly as compared with the effect of heated Laurel-leaf lard upon butterfat. The animals on the unheated-cottonseed-oil mixture averaged 197 gms. in weight at the end of 12 weeks, whereas five of the animals (one died) on the heated-cottonseed-oil mixture averaged 162 gms. at that time. This effect differed from the results shown in table 1 (Exp. W) where the fat solution (cottonseed oil-cod liver oil) had been incorporated in the basal mixture and stored at room temperature. In

this latter experiment one rat died in the fifth week, and all succumbed by the sixteenth week.

Another experiment was performed to determine the effect of heated fats upon vitamin A activity when the vitamin A source was fed separately. Control groups receiving unheated fats were used to check the animals on the corresponding heated fats. Butterfat was fed in dishes in amounts equivalent to 10 and 20 per cent of the total food consumption. The rats on the heated Laurel-leaf lard reached an average weight of 207 gms. at the end of the sixteenth week on the 10 per cent butterfat level and an average of 173 gms. at the end of the sixteenth week on the 20 per cent butterfat level compared with average values of 226 gms. and 271 gms. for the respective controls.

Three groups of rats were fed commercial lard and butterfat along with the basal mixture. The animals receiving the lard and butterfat mixed in the basal ration averaged 217 gms. at the end of 13 weeks. The animals receiving the heated lard and butterfat in the basal diet died; whereas the animals receiving heated lard in the basal mixture while butterfat was fed separately weighed 171 gms. after 13 weeks. Like results were obtained when open kettle lard, Laurel-leaf lard and Clix were employed in similar experiments with butterfat as the source of vitamin A activity. It was apparent that vitamin A activity was destroyed when this vitamin-A-bearing fat was mixed in the heated-fat ration but only partial destruction of vitamin A activity or possibly no destruction at all occurred when the vitamin-A-bearing fat was fed apart

TABLE 2. *Effect of time of heating (one-eighth inch layer of fat at 102°C.-105°C.) on the inactivating power of fats upon vitamin A potency of butterfat and cod liver oil as shown by growth rate (+ or −) and number of rats exhibiting xerophthalmia (numerals).*

Exp.	Fat	Length of heating time						Vita-min A source[a]
		0 hr.	8 hrs.	16 hrs.	24 hrs.	48 hrs.	72 hrs.	
A.	Lard (commercial) ..	+0	+0	−3	−6			B.F.
B.	Lard (open kettle) ..	+0	−5	−5	−2			B.F.
C.	Mazola	+0	+0	−0	−0			B.F.
D.	Mazola	+0	+0	−1	−1			B.F.[b]
E.	Crisco	+0	+0	−3	−5			B.F.
F.	Lard (open kettle) ..	+0	+0	+0	+0			C.L.O.
G.	Mazola	+0	+0	−0	−0			C.L.O.
H.	Coconut oil	+0			−2	−5	−1	B.F.
I.	Lard (Laurel-leaf) ..	+0			+0	+0	+0	C.L.O.
J.	Lard (Laurel-leaf) ..	+0			−6	−3	−3	B.F.

[a] B.F. stands for butterfat and C.L.O. represents cod liver oil.

[b] The bottles containing the rations in this experiment were kept in a refrigerator at 1°C. and only enough of the ration was taken out each day to feed the rats.

from the heated-fat-ration. However, heated Laurel-leaf and open kettle lards only slightly inactivated butterfat when the rations were mixed daily.

Further experiments were conducted to evaluate the effect produced by heated fats when fed to rats and the action of such fats upon vitamin A activity. Rats fed diets in which heated fats such as lard, cod liver oil, or butterfat supplied the only fat did not succumb any sooner than those that were fed on a diet free of fat and vitamin A. Whipple (8) had found that rats receiving oxidized fats grew at about the same rate as the fat-free controls and developed symptoms very similar to those of fat deficiency disease; their length of life was longer than that of the animals fed the fat-free diet.

Open kettle lard autoclaved at 15 pounds pressure for 15 minutes in a one and one-half inch layer failed to inactivate butterfat. This process failed to affect the lard in so far as its response to subsequent heat in thin layers at 102° C. to 105° C. was concerned. Lard heated at this temperature, in a layer one and one-half inches thick, did not inactivate butterfat.

Studies were also made of the effect of duration of heating at 102° C. to 105° C. upon the ability of the heated fat to inactivate cod liver oil or butterfat. The data are recorded in table 2. The response of a fat to heat treatment increased with the length of heating time. Fats varied in the degree of response to length of time of heating (compare Exps. A with B, and F with G). Although heated Mazola inactivated butterfat no more effectively than other fats heated for equivalent lengths of time (Exps. A, B, C, D, and E) it was more potent in the destruction of vitamin A activity of cod liver oil than was lard (Exps. F, G, and I). Heating fats longer than 24 hours did not alter their destructive capacity. Although heated lard failed to inactivate cod liver oil, the animals upon this fat did not grow as well as the controls which received the unheated fat. This decrease in growth regularly became more pronounced as the fat was heated for greater intervals of time until a minimum rate of growth was attained. Further heating did not reduce the rate of growth of the animals. In Exp. I, table 2, at the end of 12 weeks, the rats upon the fat heated for 24, 48 and 72 hours averaged 102, 101 and 100 gms. in weight respectively. Mazola heated for 16 hours inactivated cod liver oil; whereas lard heated for 72 hours did not inactivate this liver oil.

Previous data (1) on the effect of baking on the vitamin A content of cookies containing different fats were consistent when egg yolk was employed as the source of vitamin A, but they were contradictory and difficult to explain when butter supplied this vitamin. Consequently, a series of experiments with butter cookies was performed again. The cookies and rations were the same as given in the previous paper (1), but the baking temperature was 191°C. There was no evidence of the destruction of vitamin A activity in any of the cookies containing butter alone or butter and some other fat such as lard, Crisco or Clix.

TABLE 3. *Relation of type and concentration of anti-oxygen and kind of fat on efficacy of anti-oxygenic activity as shown by growth rate (+ or −) and number of animals exhibiting xerophthalmia (numerals)[a].*

Exp.	Anti-oxygen	Fat	Unheated				Heated							
			0	*1*	*5*	*10*	*0*	*0.025*	*0.1*	*0.5*	*1*	*5*	*10*	*20*
A.	Wheat germ oil	lard (open kettle)	+0			+0	−2				−1	−6	−2	−5
B.	Egg oil	lard (open kettle)	+0				−2				−3	−2	−2	+0
C.	Soya flour	lard (Laurel-leaf)	+0				−1						−3	
D.	Fuller's earth	lard (Laurel-leaf)	+0			+0	−1						−5	
E.	Egg yolk	lard (open kettle)	+0			+0	−2						−5	
F.	Thymol	lard (open kettle)	+0	+0			−2	−4		+0	+0			
G.	Thymol	Mazola	+0	+0			−0		−3	+0	−0			
H.	Thymol	Mazola	+0	+0	+0		−0				−2	−4		
I.	Thymol	Soybean oil	+0	+0	+0		−0				−0	−4		

[a] The italicized figures represent the percentage of anti-oxygen in the fat. The fat solution of the anti-oxygen comprised ten per cent of the diet. A one-eighth-inch layer of the material (fat or fat anti-oxygen solution or mixture) was heated at 102°C. to 105°C. for 24 hours.

The fats in table 3 were also used in experiments of table 1.

Butterfat was used as a source of vitamin A in these experiments.

Experiments were next conducted with anti-oxygens. The data are recorded in table 3. Anderegg and Nelson (5) had shown in 1926 that wheat germ oil protects cod liver oil in skimmed milk powder diets. These authors stated: "Addition of ethly alcohol, wheat oil, or water to such mixtures exerts a protective action. Skimmed milk powder diets, upon which rats are sterile, were so changed by the addition of water and administering the cod liver oil separately that fourth generation young have now been obtained." Mattill (9) verified these results and also showed that the susceptibility of butter to oxidation was decreased by a trace of wheat germ oil. The results of their experiments led Anderegg and Nelson (5) to believe at that time that the existence of vitamin E was highly improbable. Because of the protective action of wheat germ oil as shown by the experiments of Anderegg and Nelson (5), it was believed that this oil might prevent the production of dele-terious properties in lard when subjected to heat. In our own ex-periments it was found that wheat germ oil in 20 per cent concentration was ineffective as an anti-oxygen with lard. However, it possessed anti-oxygenic properties even at a 10 per cent level under less drastic oxidative conditions (table 4). Experiment B, table 3, showed that egg oil was ineffective at a concentration of 10 per cent but was effective at a level of 20 per cent. Soya flour, fuller's earth, and egg yolk in 10 per cent concentrations failed to protect lard against the heat treatment. Thymol was an effective anti-oxygen with lard in a 0.5 per cent con-

TABLE 4. *The effect of the length of heating time upon the anti-oxygenic activity of wheat germ oil*[a]

	0 hours	6 hours	12 hours	18 hours	24 hours
I. Lard	242 (15)	210 (15)	97 (6-9) 5	61 (6-9) 6	69 (5-10) 2
II. Lard-wheat germ oil solution	237 (15)	251 (15)	223 (15)	61 (6-9) 6	74 (6- 9) 2

[a] The lard-wheat germ oil solution consisted of 90 per cent lard and 10 per cent wheat germ oil. The three groups of rats upon lard I-6, I-12 and I-18 received 90 gms. of the heated lard whereas all the other groups received 100 gms. of fat. These three groups received an additional 10 gms. of dextrin.

The numbers in tables 4, 8, 9, 10, 11, 12 and 13 have the following meaning: An expression which contains parentheses enclosing only a single number indicates that all of the rats survived and grew. This single number represents the length of the experiment in weeks, and the number preceding the parentheses represents the average weight of the six rats at the termination of the experiment, for example, 178 (11) signifies that the average weight of the animals at the end of the eleventh week was 178 gms. An expression which contains parentheses enclosing two num-bers joined by a hyphen indicates that all of the rats died. The number preceding the parentheses represents the average weight of the rats at the last weighing be-fore death, and the enclosed numbers give the weeks in which the first and last deaths occurred; the digit following the parentheses refers to the number of animals which exhibited xerophthalmia. For example, 62(6-8)5 means that all the rats died, that their average weight at the last weighing prior to death was 62 gms., that the first death occurred in the sixth week and the last one in the eighth week, and that five rats showed symptoms of xerophthalmia.

In the experiments of table 4, butterfat was used as a source of vitamin A.

centration (Exp. F) but five per cent was insufficent tò protect either Mazola or soybean oil against heat (Exps. H and I). It may be observed that Mazola and soybean oil were not protected by five per cent of thymol while lard with a lower iodine number was protected against heat by a concentration one-tenth as great (0.5 per cent). The protective action of thymol with lard was diminshed by extending the heating time; whereas the rats fed the one per cent lard solution of thymol which had been heated for 24 hours had an average weight of 224 gms. at the end of the twelfth week, the rats receiving a similar solution which had been heated for one week averaged only 156 gms. at the end of the same period.

Experiments were made to ascertain whether or not heated fats were toxic. Fridericia (3) and Powick (10) had concluded from feeding experiments that heated fats were not toxic. Fats were treated like those employed in the feeding tests, that is, they were maintained in a layer, one-eighth inch thick, for 24 hours at 102° C. to 105° C. The fats either at room temperature or at the minimum temperature necessary to maintain a liquid condition were injected intraperitoneally into rats weighing

TABLE 5. *Effect of heat upon the toxicity of fats with and without thymol as shown by the number of rats that died in the week after the injection*[a]

Exp.	Fat	Percentage thymol	Unheated 10 cc.[b]	Heated[c]				
				10 cc.	8 cc.	6 cc.	4 cc.	2 cc.
1.	Mazola	0	0	9	10	9	3	
2.	Mazola	1	0	9	1			
3.	Mazola	5	10	6				
4.	Soybean oil	0	0	7	2	4		
5.	Soybean oil	5		3				
6.	Cod liver oil	0	0	10	10	10	10	2
7.	Cod liver oil	1	0	10	10	10	8	1
8.	Lard (open kettle)	0	0	9	10	8	3	
9.	Lard (open kettle)	1	2	4				
10.	Coconut oil	0	0	10				
11.	Peanut oil (rancid)	0	2	9	7	6		
12.	Butterfat	0	0	6	6	6	3	
13.	Cottonseed oil	0	0	10	10	5		
14.	Lard (Laurel-leaf)	0	0	10	8	4	0	
15.	Olive oil	0	0	6	0	1	3	'

[a] Ten rats were employed at each level of fat injection.
[b] Number of cc. of fat or fat-thymol solution that was injected.
[c] A one-eighth-inch layer of the fat was heated at 102°C.-105°C. for 24 hours.

between 200 and 300 gms. The animals were given the usual growing ration which has been used successfully in this laboratory for many years. The number of rats which succumbed in one week was taken as a measure of the toxicity of the fats. The data are given in table 5. The control animals received an intraperitoneal injection of 10 cc. of unheated fat; the heated fat was injected at levels of 10 cc., eight cc., six cc., four cc., and two cc. Ten rats were employed at each level of fat injection. The data showed that heated fats were toxic to rats and in some cases markedly so. Unheated fats were not toxic to rats, but a sample of peanut oil which was definitely rancid, organoleptically and by the Kreis test, proved slightly toxic. The toxicity of this rancid peanut oil was markedly increased by heating. Since thymol proved a good anti-oxygen with lard, tests were made with this substance to determine its effect upon the development of toxicity by heat in fats. The thymol was dissolved in the fat, and the fat-thymol solution was heated in a thin layer at 102° C. to 105° C. for 24 hours. The control animals received unheated fat-thymol solutions; whereas the others received the corresponding heated fat-thymol solutions. A one per cent solution of thymol in lard was slightly toxic, but the same concentration of thymol in Mazola and cod liver oil was not toxic. All of the rats receiving 10 cc. of a five per cent solution of thymol in Mazola died within fifteen minutes after the injection. One per cent of thymol in fats seemed to have a slight protective action against heat. Heated and unheated solutions of one per cent of thymol in lard were non-toxic when employed in the feeding experiments (Exp. F, table 3). The unheated solutions of five per cent thymol in soybean oil and in Mazola were non-toxic when fed (Exps. H and I, table 3).

Experiments were performed to determine the toxicity of stored fats. The results are recorded in table 6. Mazola and lard which had been stored at room temperature were definitely rancid (positive Kreis test), but were not toxic when injected intraperitoneally; fats may be definitely rancid as determined by the Kreis test and yet be non-toxic to rats. All of the fats gave a positive Kreis test following storage at room temperature (24° C. to 30° C.), but of the fats stored in the refrigerator (1° C.) cod liver oil alone reacted positively to this test. Cod liver oil, stored either at room temperature or at refrigerator temperature, was toxic to rats while none of the other fats (lard, butter-fat, Mazola) similarly stored proved toxic to the animals.

The difference in unsaturation of corn oil and cod liver oil hardly seemed great enough to account for their different response to storage. However, the types of acids which account for the unsaturation in the two fats are different; oleic and linoleic acids are responsible for most of the unsaturation of Mazola while cod liver oil contains in addition to these acids other highly unstable acids. Corn oil contains inhibitols while cod liver oil is practically devoid of such substances. Thus, although the susceptibility of a fat to oxidation was somewhat dependent

TABLE 6. *Effect of storage upon toxicity of different fats as shown by rats that died in the week after injection*[a]

Fat	Stored at 1°C. 10 cc.[b]	Stored at 24°-30°C. 10 cc.	4 cc.	2 cc.	Number of days stored
Cod liver oil	10+[c]				479
		10+			462
			8+		550
				2+	562
Mazola	0—	.			496
		0+			514
Lard	0—				540
		0+			520
Butterfat	0—				494
		0+[d]			495

[a] Ten rats weighing between 200 and 300 gms. were employed at each level of injection.

[b] The number of cc. which were injected intraperitoneally into the rats.

[c] The + (positive) and — (negative) signs refer to the reaction of the fats to the Kreis test The Kreis test was conducted upon a 1:19 solution of the fats in mineral oil.

[d] The Kreis test in this fat was only very slightly positive.

upon its degree of unsaturation, it was markedly affected by certain other properties of the fat such as the kind of acids responsible for the unsaturation and the presence of anti-oxygens. In cod liver oil, both of these factors, instability of the constituents and lack of anti-oxygens, were responsible for the rapid decomposition of the oil. Lard, although it contained almost no inhibitols, was less easily decomposed because its fatty acids were fairly stable. Mazola, although highly unsaturated, contained sufficient inhibitols to preserve it. The oxidative conditions in the heating experiments were sufficiently drastic to mask these differences; all four of the fats (lard, Mazola, butterfat, cod liver oil) when heated inactivated butterfat, and both heated Mazola and heated cod liver oil inactivated cod liver oil. This was to be expected since Mazola is highly unsaturated; thus as soon as the anti-oxygenic activity was destroyed by heat, Mazola became sufficiently oxidized to inactivate cod liver oil. Butterfat and lard failed to inactivate cod liver oil, when similarly heated, because they are far more saturated than the corn oil.

It was decided to subject the two highly unsaturated fats, Mazola and cod liver oil, to other mild oxidative conditions to obtain further information about the factors involved in determining the susceptibility of fat to oxidation. The fats were oxidized by bubbling a stream of dry air through them at room temperature, and then the oxidized fats were

injected intraperitoneally into rats. Air was dried by bubbling it through concentrated sulfuric acid.

Dry air oxidized cod liver oil far more readily than it oxidized Mazola; the latter oil, aerated for one week, did not turn rancid (negative Kreis test) or become toxic; whereas the former oil, when aerated for this length of time, became definitely rancid (positive Kreis test) and toxic. Apparently the mild oxidative measures were sufficient to affect the unstable acids found in fish liver oils but failed to oxidize the unsaturated acids present in Mazola. The data supplement the results of the previous experiments upon the toxicity of stored fats. Cady and Luck (11) observed that sulfur dioxide rapidly and completely inactivated cod liver oil but did not affect the vitamin A activity of alfalfa and only lessened the vitamin A activity of butterfat. It was therefore evident that different sources of vitamin A activity responded differently to the same chemical treatment. The vitamin A activity of cod liver oil was far more resistant to the destructive action of heated fats but was destroyed much more easily by finely divided solids than was the vitamin A activity of butterfat.

Experiments were performed to determine the effect of heat upon the esters of oleic acid. The methyl and ethyl esters of this acid were heated and then tested for toxicity by intraperitoneal injection into rats weighing between 200 and 300 gms. Ten rats were employed at each level of injection. Following the injection the rats were maintained for a week upon the growing ration and the number of animals which died in this period was taken as a measure of toxicity. The results are recorded in table 7. The esters were also tested for rancidity by the Kreis test. The esters gave a negative Kreis test but reacted positively to this test following heating. Whereas injections of 10 cc. of the esters were not toxic to the rats, injections of one cc. of the heated esters proved definitely toxic. The heated esters were far more toxic than the heated fats (table 5).

Whereas all the rats injected with 10 cc. of heated Mazola suc-

TABLE 7. *Effect of heat upon toxicity of esters of oleic acid as shown by the number of animals that died in the week after injection*[a]

	Number of rats dead in one week			
	Unheated	Heated[b]		
Ester	10 cc.	2 cc.	1 cc.	0.4 cc.[c]
Methyl oleate	0—	9+	7+	0+
Ethyl oleate	0—		9+	

[a] The + (positive) and — (negative) signs refer to the reaction of the esters to the Kreis test. This test was conducted upon a 1:19 solution of the esters in mineral oil. Ten rats weighing between 200 and 300 gms. were injected at each level.

[b] Heated—maintained at 102°C.-105°C. in a one-eighth-inch layer for twenty-four hours.

[c] The number of cc. which was injected intraperitoneally.

cumbed, only four of the animals died when the same amount of this heated oil was given by way of stomach tube. A similar result was obtained with heated ethyl oleate; one cc. killed the rats upon intraperitoneal injection, but two cc. failed to kill any of the animals when introduced by way of stomach tube. The rats receiving heated Mazola or heated ethyl oleate by way of stomach tube invariably had a portion of their stomach wall discolored but were otherwise normal. The death of the four animals receiving the heated Mazola by way of stomach tube was not brought about by the volume of the oil since the rats given an equal amount of the unheated Mazola by the same process remained alive and were normal in all respects.

Experiments were conducted to ascertain if regeneration of the heated fats was possible. Fiero (12) had renovated rancid coconut oil by agitation with clay but had failed to produce a similar result in rancid lard. Experiments were therefore performed to determine whether stirring heated Mazola with fuller's earth would regenerate the fat. Fuller's earth was added to a number of portions of heated Mazola in an amount equal to one-tenth of the weight of the fat. The mixtures were stirred by motor for three hours and 30 hours, filtered, and the filtrate employed in the rations. Both the heated and unheated fats were treated in the same manner. The ration fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat, and 100 gms. of the filtered Mazola. Stirring heated Mazola for 30 hours with fuller's earth failed to regenerate the fat; the rats fed this fat exhibited vitamin A deficiency. Heated Mazola stirred for three hours with fuller's earth was still toxic when injected intraperitoneally into rats.

Lease, Lease, Weber, and Steenbock (13) lowered the inactivating potency of rancid fats by heating them in an atmosphere of nitrogen. However, experiments H and J, table 2, indicate that further heating of heated fats in air failed to reduce their inactivating power.

Roschen and Newton (14) restored the palatability of rancid lard by blowing steam through it and thus removing the volatile products of oxidation, which were responsible for the rancid odor and taste. An attempt was made to regenerate heated Mazola by steam distillation. The heated and unheated fats were steam distilled for two hours. The fat was decanted, filtered, and the filtrate employed for the tests. Steam distillation failed to regenerate heated Mazola; the heated fat inactivated butterfat after the steam distillation as well as it did before the treatment.

Stephan (15) derancidified fats by treating them with semicarbazide hydrochloride ($NH_2 \cdot CO \cdot NH \cdot NH_2 \cdot HCl$) and filtering them. The filtrate gave a negative Kreis test. An attempt was made to regenerate heated fats by this method. The heated fat, Mazola, was stirred with one gm. of semicarbazide hydrochloride and 0.4 gm. of sodium stearate per 100 gms. of fat for 10 hours at 50° C. The fat was then filtered. The filtrate still gave a positive Kreis test and was toxic to rats when injected intraperitoneally.

A sample of Mazola that had become rancid upon standing at room temperature also remained positive to the Kreis test after treatment with semicarbazide hydrochloride. This latter fat was non-toxic both before and after the treatment. In another experiment the treatment with semicarbazide hydrochloride was modified by stirring the mixture at the boiling temperature of water for 10 hours. Following this treatment heated Mazola gave a negative Kreis test and was non-toxic when injected intraperitoneally into rats. Heated cod liver oil also reacted negatively to the Kreis test following the treatment with semicarbazide hydrochloride at 100° C., but it was still somewhat toxic to rats when injected intraperitoneally. Apparently the treatment reduced the toxicity of the heated cod liver oil considerably. Both heated Mazola and heated cod liver oil became very viscous upon being treated with the semicarbazide hydrochloride at 100° C. The modified semicarbazide hydrochloride treatment did not destroy the vitamin-A-inactivating power of either of the heated oils. It was thus apparent that the substances responsible for the toxic properties of heated fats were different from the substances responsible for the ability of these fats to inactivate vitamin-A-bearing oils. The former are aldehydes or ketones while the latter are neither aldehydic nor ketonic.

Experiments were made to determine the effect of feeding soybeans to hogs upon the susceptibility of the lard to oxidation and rancidity. A number of lards prepared from hogs which had been fed varying amounts of soybeans were supplied by Professor F. J. Beard of the Animal Husbandry Department. The lards were tested on rats. The "soybean" lards were fed to rats to determine whether the lards from hogs fed rations high in soybeans (16) were any more susceptible to oxidation and rancidity than the lards from the hogs receiving diets containing little or no soybeans. The ration fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat and 100 gms. of the lard. The data are recorded in table 8.

TABLE 8. *Growth of rats fed lards (heated in a one-eighth-inch layer at 102°C.-105°C. for 24 hours) from soy-bean fed hogs*

| | Weight of rats in grams | | Iodine value of the lard |
Diet of hogs[b]	Unheated	Heated	
1. No protein supplement	229(12)[a]		68.87
2. Meat meal tankage	217(12)		67.91
3. Cracked soybeans	220(12)		78.76
4. Grain mixture A	216(12)		72.98
(5 percent cracked soybeans)			
5. Grain mixture B	223(12)		79.99
(10 percent cracked soybeans)			
6. Grain mixture C	205(12)	54(5-6)5	85.25
(20 percent cracked soybeans)			
7. Grain mixture D	231(12)	63(4-6)3	71.92
(10 percent soybean oilmeal)			
8. Grain mixture E (9.9 percent soybean oilmeal + 1.3 percent soybean oil)	195(12)	57(5-5)5	79.17
9. Same as number 1	226(12)		

[a] For the meaning of the numbers see explanation to table 4.
[b] Details given in Animal Husbandry Leaflet No. 150, Iowa State College.

TABLE 9. *The effect of temperature and duration of storage upon open kettle lard*

Exp.	Storage treatment	Weight of rats
1.	Control	221 (12)[a]
2.	Stored at 25°C. for one week	203 (12)[b]
3.	Stored at 25°C. for two weeks	220 (12)
4.	Stored at 25°C. for four weeks	203 (12)
5.	Stored at 39°C. for one week	226 (12)
6.	Stored at 39°C. for two weeks	225 (12)
7.	Stored at 39°C. for four weeks	201 (12)

[a] For the meaning of the numbers see explanation to table 4.
[b] Average weight of five rats. (One rat died.)

Whereas Lea (17) had found that the susceptibility of lard to oxidation differed greatly even when changes in iodine value were scarcely discernible, the results obtained in the experiments recorded in table 8 indicated that, although the iodine values of lard increased, the susceptibility of the fat to oxidation when mixed in the ration was not increased significantly. It is to be understood that only one kilogram of ration was prepared at a time and when this was consumed another kilogram quantity was prepared, etc. Although lard number 8, as well as the mixed ration containing this lard, tended to become rancid, the increased susceptibility of this fat to oxidation cannot be attributed solely to its greater unsaturation since this lard did not possess the highest iodine value. Thus although soybean oil and cod liver oil are of a somewhat similar degree of unsaturation, a change in iodine value of lard brought about by feeding soybeans did not affect the susceptibility of the fat to oxidation as much as a change brought about by feeding cod liver oil as reported by Lea (17). Therefore the susceptibility of lard to oxidation depended upon other factors besides unsaturation. The results obtained with the heated lards (Exps. 6, 7, and 8, table 8) did not shed any light on the matter of susceptibility to oxidation since the treatment which was employed proved sufficiently drastic to produce inactivating ability in all the three fats tested.

A study was made of the effect of temperature and duration of storage on lard. Wilson's open kettle lard was stored under the conditions described in table 9. Following this treatment the fat was placed in a refrig-

TABLE 10. *Effect of various concentrations of thymol in preserving open kettle lard*

Exp.	Fat-thymol solutions	Average weight of rats in grams	Time of storage at 24°C.-30°C. in days
A	Lard	217 (12)[a]	525
B₁	Lard containing 1 percent thymol	216 (12)	525
B₂	Lard containing 0.5 percent thymol	199 (12)	529
B₃	Lard containing 0.1 percent thymol	197 (12)	529
B₄	Lard containing 0.025 percent thymol	170 (12)	529
C	Lard	204 (12)	529

[a] For meaning of the numbers see explanation to table 4.

TABLE 11. *Effect of aerated fats upon vitamin A activity of butterfat*[a]

Fat	Average weight of rats in grams	
	Unaerated	Aerated[b]
Mazola	230 (12)	231 (12) [c]
Cod liver oil	195 (12)	178 (12)

[a] For the meaning of the numbers see footnote to table 4.

[b] Aerated—a steady stream of dry air (passed through conc. H_2SO_4) was bubbled through the fat for 24 hours at room temperature.

[c] Average of five rats.

erator at the same temperature as that employed in storing the control portion, namely 1° C. The ration fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat and 100 gms of lard. The results are given in table 9. The lard that had been stored for four weeks at 39° C. did not have a detrimental effect upon the vitamin A activity of butterfat when these two fats were melted, mixed, and the mixture incorporated in the ration.

A study was made of the preservative action of thymol for lard stored at room temperature (24°C. to 30°C.). The fat (Wilson's open kettle lard) was melted and divided into a number of portions into each of which a definite weight of thymol was dissolved by stirring. The concentrations of the solutions are designated in table 10 wherein the data are recorded. The solutions were then stored in closed glass containers at room temperature.

TABLE 12. *Time of anti-oxygenic action relative to heat treatment of open kettle lard shown by weight of rats*[a]

Exp. No.	Substance tested	Average weight of rats in grams
1.	Lard-thymol solution[b]	215 (12)
2.	Heated lard-thymol solution	224 (12)
3.	Heated lard	69 (5-10) 2
4.	Heated lard plus one percent thymol	93 (5-17) 4

[a] For the meaning of these numbers see footnote to table 4.

[b] A one-eighth-inch layer of the fat or fat-thymol solution was heated at 102°C.-105°C. for 24 hours. The lard-thymol solution consisted of 99 per cent Wilson's open kettle lard and one per cent thymol. In Experiment 4 the thymol was added after the heat treatment.

The ration fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat and 100 gms. of lard or lard solution. The lard used in experiment C was stored at 1° C. during the entire time. The nutritive value of the lard, evaluated by feeding the fat to rats in the above described diet, was not diminished by storing it in closed glass containers at room temperature for 525 days (Exp. A, table 10). The melting and stirring process employed to dissolve thymol in the lard rendered the fat slightly more

TABLE 13. *Effect of water upon the destructive power of heated open kettle lard*[a]

Exp. No.	Quantities of lard and water	Average weight of rats in grams	
		Unheated	Heated
1.	100 gms. lard	238(14)	69(5–10)2
2.	100 gms. lard and 100 cc. water	214(14)	71(6–14)3

[a] For the meaning of these numbers see footnote to table 4. A one-eighth-inch layer of the lard was heated at 102°C.-105°C. for 24 hours.

susceptible to oxidation during subsequent storage, because the rats fed the lards containing 0.5 per cent, 0.1 per cent, and 0.025 per cent of thymol (Exps. B_2, B_3, and B_4) did not grow so well as those receiving the lard stored without any thymol. This latter fat had not been subjected to either melting or stirring prior to storage. One per cent concentration of thymol proved sufficient to protect the lard against the oxidation induced by stirring and melting (Exp. B_1); the rats receiving this lard grew as well as those fed the unstirred and unmelted lard in Experiment A.

Experiments were conducted to ascertain the effect of aeration upon

TABLE 14. *Effect of heat and storage on the quantity of free fatty acids*[a]

Fat	No. hours heating	No days stored	Free fatty acids[b]	
			Before storage	After storage
1. Lard (commercial)	0	88	0.28	0.55
	8	55	0.49	0.56
	16	46	1.01	1.08
	24	55	3.58	3.63
2. Lard (open kettle Wilson)	0	85	0.33	0.32
	8	79	0.61	1.79
	16	85	1.84	3.09
	24	85	1.51	3.31
3. Lard (Laurel-leaf Wilson)	0	86	0.46	0.64
	8	80	1.07	2.19
	16	85	1.82	2.50
	24	85	2.49	3.90
4. Mazola	0	50	0.38	0.26
	8	50	0.41	0.70
	16	46	1.97	2.22
	24	46	2.44	3.25

[a] Fats were heated by maintaining a one-eighth-inch layer at 102°C.-105°C. Storage temperature was 1°C.

[b] The free fatty acids are expressed in percentage as oleic acid.

fats as determined by the subsequent action of the aerated fats on the vitamin A activity of a properly balanced diet. A steady stream of air, dried by being passed through concentrated sulfuric acid, was bubbled through the fat at room temperature for 24 hours. The ration fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat and 100 gms. of the fat under investigation. The results are given in table 11. Aerated Mazola and aerated cod liver oil failed to inactivate butterfat. Both the aerated and non-aerated fats responded negatively to the Kreis test.

Experiments were made to determine whether thymol lengthened the induction period of lard or whether it acted after the termination of this period. One per cent concentration of thymol was employed with Wilson's open kettle lard. In one case the anti-oxygen was added to the lard prior to heating and in the other it was added to the fat following this treatment. The ration consisted of the normal basal mixure plus 100 gms. of butterfat and 100 gms. of the lard-thymol solution. The results are given in table 12 and are compared with data obtained by feeding the heated lard with no thymol.

Whereas one per cent of thymol in lard when added prior to the heat

TABLE 15. *Effect of heat and storage upon the iodine number of fats*[a]

Fat	No. hours heating	No. days stored	Iodine number[b]	
			Before storage	After storage
1. Lard (commercial)	0	96	61 5	61.5
	8	51	60.4	58 8
	16	51	57.3	54 2
	24	46	42.0	39.4
2. Lard (open kettle)	0	85	78.1	64.6
	8	79	75.1	61 8
	16	85	70.4	54 4
	24	85	73.3	54.7
3. Lard (Laurel-leaf Wilson)	0	86	81.2	68.3
	8	80	79.1	63.3
	16	85	74.7	59.2
	24	85	73 0	51.7
4 Mazola	0	50	127.1	124 5
	8	50	125.8	118.2
	16	46	95 7	98 3
	24	46	92.4	95 5

[a] The storage temperature was 1°C. Fats were heated in a one-eighth-inch layer at 102°C.-105°C

[b] Iodine number is the number of gm of iodine absorbed by 100 gms. of fat.

TABLE 16. *Effect of heat and storage upon the peroxide value of fats*[a]

Fat	No. hours heating	No. days stored	Peroxide value[b]	
			Before storage	After storage
1. Lard (commercial)	0	88	3.3	5.0
	8	55	136.8	205.0
	16	51	249.0	198.0
	24	55	133.0	162.0
2. Lard (open kettle Wilson)	0	85	1.7	4.4
	8	79	108.0	282.0
	16	85	292.0	290.0
	24	85	305.0	299.0
3. Lard (Laurel-leaf Wilson)	0	86	4.5	5.8
	8	80	197.0	323.0
	16	85	313.0	250.0
	24	85	295.0	328.0
4. Mazola	0	50	0.7	5.8
	8	50	148.0	178.0
	16	46	156.0	310.0
	24	46	152.0	280.0

[a] Fats were heated in a one-eighth-in. h layer at 102°C.-105°C. Storage temperature was 1°C.
[b] Peroxide value given in milli-equivalents per 1000 gms. of fat.

treatment effectively prevented inactivation of butterfat by the heated lard (Exps. 2 and 3), it only delayed the inactivation when added to the lard following the heat treatment (Exps. 3 and 4). It was thus apparent that this anti-oxygen acted by inhibiting the oxidation of the lard, protecting the induction period against the action of heat. It did not destroy the inactivating properties of heated lard but delayed the destruction of vitamin A activity in butterfat (Exps. 3 and 4).

Anderegg and Nelson (5) and Marcus (7) discovered that water mixed into the diet protected cod liver oil against the destructive action of highly desiccated solids. Experiments were conducted to determine whether the presence of water in the diet would alter the action of heated fats upon vitamin A activity. The diet fed the rats consisted of the normal basal mixture plus 100 gms. of butterfat, 100 gms. of Wilson's open kettle lard, and 100 cc. of tap water. The results of the experiments are given in table 13 and are compared with data of experiments in which the rats received the same diet except for the water.

The presence of water in the ration caused little, if any, delay in the destructive action of heated lard on the vitamin A activity of butterfat.

The quantity of free fatty acids in a fat increased with the duration of heat treatment and storage (table 14), while the iodine number of a

fat decreased as the heating and storing progressed (table 15). This may well be expected since heat as well as storage oxidized and hydrolyzed the fat. The hydrolysis set free the constituent fatty acids; the oxidation occurred at the double bonds reducing the iodine number. There was a tendency for the peroxides in the fat as heating progressed to reach a maximum concentration and then to decrease in quantity (table 16). They appeared to be unstable and may decompose upon further heating.

SUMMARY

1. Most of the heated fats that were studied inactivated butterfat; the heated fat in contact with the source of the vitamin caused the inactivation. All of the oils with iodine numbers corresponding to those of semi-drying or drying oils, upon being heated, inactivated cod liver oil as well as butterfat; all the fats or oils with lower iodine numbers, upon being similarly heated, failed to inactivate cod liver oil but, with the exception of one heated coconut oil sample, inactivated butterfat. The vitamin A activity of cod liver oil was found to be far more resistant to the destructive action of heated fats but much more easily destroyed by finely divided solids than was the vitamin A activity of butterfat.

2. The saturated fatty acids, stearic and palmitic, and the glycerol portion of fat were not responsible for the inactivating potency of heated fats; the unsaturated fatty acids, for example, oleic, linoleic, and linolenic, were involved in the response of fats to heat.

3. The destruction of vitamin A activity in heated-fat diets was apparently not brought about by the heated fat alone; the decomposiion products of the vitamin-A-containing oil also participated.

4. The response of fats to heat, that is, the inactivating power of heated fats, increased with the surface exposed to air and with the length of the heat treatment; the rate at which the inactivating ability developed varied with the fat employed. The vitamin-A-inactivating power of heated fats was not produced by heat alone but by the combined action of heat and oxygen; heat acted mainly to accelerate the action of oxygen.

5. Autoclaved lard, most of the stored fats, and aerated fats did not destroy vitamin A activity.

6. Water incorporated in the ration did not protect vitamin A activity against the action of heated fats.

7. Heated fats did not appear to be toxic when ingested but were toxic when injected intraperitoneally; unheated fats were not toxic either when ingested or injected.

8. Anti-oxygens varied in their efficacy with the fat employed; thymol was an excellent anti-oxygen with lard but did not possess antioxygenic properties with Mazola or soybean oil. Anti-oxygens preserved the induction period and were ineffective if added after the termination of this period. Their activity diminished as oxidative conditions were intensified.

9. Baking did not destroy the vitamin A activity of butter in cookies; this was true whether the cookies contained butter as the only fat or butter and some other fat such as lard, Clix, or Crisco.

10. Soybeans in the diet of hogs, although they increased the iodine number of the hog fat, did not markedly increase the susceptibility of the lard to oxidation.

11. The susceptibility of an oil to oxidation was found to be dependent upon the degree of unsaturation, the type of acid responsible for the unsaturation, and the kind and concentration of anti-oxygens in the fat.

12. Some aerated fats and some stored fats were toxic when injected intraperitoneally; others were not. The results indicated that fats may give a positive Kreis test and yet be non-toxic when injected intraperitoneally.

13. Although heated Mazola and heated ethyl oleate were definitely toxic when injected intraperitoneally, they proved only slightly injurious when introduced by way of stomach tube.

14. Heated Mazola was not regenerated by agitation with fuller's earth or by steam distillation. Semicarbazide hydrochloride detoxified heated fats but failed to reduce their inactivating properties; the toxic substances are either aldehydes or ketones while the substances responsible for the inactivation are neither aldehydic nor ketonic.

15. The free fatty acids in the fats increased and the iodine number decreased upon heating and/or storing.

16. There was a tendency for the peroxides in a fat to increase to a maximum and then to decrease upon heating and/or storing. The inactivating potency of the heated fats was not strictly proportional to their peroxide values.

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NOTE ON THE DEGRADATION OF PROPIONIC ACID SYNTHE-SIZED BY PROPIONIBACTERIUM*

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Recently Carson et al. (1940) have published results which are of considerable interest. They have investigated the fermentation of glycerol by propionic acid bacteria using radioactive carbon (C^{11}) to label the CO_2. For several years it has been known that these heterotrophic bacteria can assimilate CO_2 (Wood and Werkman, 1935). The mechanism by which this assimilation is accomplished has assumed considerable importance because it may offer some information relative to assimilation in autotrophic photosynthesis and chemosynthesis. Particularly the results of Carson et al. emphasize this view because they report that CO_2 fixed in the product, propionic acid, is in all three carbons of the molecule. This implies that the bacteria build up the molecule entirely from 1-carbon compounds just as in autotrophism. The evidence was obtained by degrading the biologically formed acid and locating the radioactive carbon in the fragments of the molecule. This was accomplished by decarboxylating the barium salt with heat to yield barium carbonate and diethyl ketone, and by a second method of alkaline permanganate oxidation to yield oxalic acid and carbonate. The C^{11} was found in all three products and in proportions indicating a general distribution of C^{11} in the chain.

Wood and Werkman (1938, 1940) and Wood et al. (1940) presented evidence that CO_2 is assimilated in this fermentation by the following reaction: $CO_2 + CH_3.CO.COOH = COOH.CH_2.CO.COOH$. Succinic acid may then be formed by reduction of the oxaloacetic acid and propionic acid by decarboxylation of a dicarboxylic acid, perhaps succinic acid. Accordingly the fixed CO_2 would be located in the carboxyl group of the propionic acid.

Because of the theoretical importance of the results of Carson et al., and disagreement with our proposals, we have reinvestigated the problem by degrading propionic acid obtained from glycerol fermentations in which the stable isotope of carbon (C^{13}) was used as a "tracer" of fixed CO_2. The propionic acid was degraded by α-bromination, conversion of the α-bromopropionic acid to lactic acid with AgOH, then oxidation of the resulting lactic acid with permanganate to acetaldehyde and CO_2.

[213]

The acetaldehyde arises from the α and β carbons and the CO_2 from the carboxyl group of the propionic acid. All the fixed C^{13} was found in the CO_2 fraction. The results, therefore, indicate, contrary to those of Carson et al., that all the fixed CO_2 is located in the carboxyl group of propionic acid. The discrepancy between the two investigations is caused either by use of unreliable degradation methods or the bacteria fixed CO_2 differently under the respective experimental conditions.

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HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES.
II. SULFIDES, SULFOXIDES, AND SULFONES[1]

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During the course of investigations in these laboratories concerned with the utilization of animal by-products, a number of long-chained aliphatic sulfur compounds have been prepared. These include the even-chained *n*-alkyl sulfides from dodecyl to octadecyl and the corresponding sulfoxides and sulfones. The sulfides were prepared by the action of alcoholic sodium sulfide on the corresponding alkyl bromides. The method is remarkably simple, and it is surprising that none of the symmetrical *n*-alkyl sulfides above octyl have been described. A U. S. patent[2] refers to the production of aliphatic sulfides by the interaction of an inorganic sulfide and straight-chained alkyl halides of ten to fifteen carbon atoms. Evidently the sulfides between decyl and pentadecyl have been prepared under this patent, but their description appears to have been withheld. No reference is given to the corresponding sulfoxides and sulfones. Cetyl sulfide was prepared by Fridau[3] in 1852, but the sulfoxide and sulfone were not described. v. Pieverling[4] attempted to prepare mellisyl (C_{31}) sulfide but obtained the mercaptan instead. Jones and Reid[5] have prepared a number of long-chained, unsymmetrically substituted sulfides by the addition of mercaptans to unsaturated hydrocarbons and have proved their structure by synthesis from the potassium salt of mercaptans and the normal alkyl halides.

A great variety of oxidizing agents has been used to convert sulfides to the corresponding sulfoxides and sulfones. Grabowsky[6] oxidized butyl sulfide to the sulfoxide with dilute nitric acid. When fuming nitric acid was used, butyl sulfone was the product.

Hinsberg[7] treated a number of sulfides and disulfides with the calculated quantities of hydrogen peroxide in acetic acid and was able to obtain the corresponding sulfoxides and disulfoxides. Hydrogen peroxide in acetone was used by Gazdar and Smiles[8] to produce sulfoxides. These investigators concurred with Hinsberg in the observation that the oxidation of sulfides with hydrogen peroxide may be readily controlled at ordinary temperature so as to proceed only to the sulfoxide stage. Excess peroxide at elevated temperature yields sulfones.

[1] The first paper in this series is: Gilman and Ford, *Iowa State College J. Sci.* **13**, 135, (1939).

[2] U. S. patent, 2,085,452, [*C. A.*, **31**, 5812 (1937)].

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[5] Jones and Reid, *J. Am. Chem. Soc.*, **60**, 2452 (1938).

[6] Grabowsky, *Ann.*, **175**, 348 (1875).

[7] Hinsberg, *Ber.*, **41**, 2836 (1908).

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Chromic acid in acetic acid solution was declared by Knoll[9] to be an excellent reagent for the oxidation of sulfides to sulfoxides. Our experience has shown that in the presence of a considerable excess of chromic acid, sulfones may also be produced.

Fries and Vogt[10] have used moist chlorine or bromine to form sulfoxides. The intermediate thio ether dichloride or dibromide is first formed and this then undergoes hydrolysis to the sulfoxide.

Bost and co-workers[11] prepared long-chained alkyl 2,4-dinitrophenyl thio ethers and oxidized these to sulfones, using potassium permanganate in acid solution.

Sodium hypochlorite solution has been used by Woods and Travis[12] in the preparation of aliphatic and aromatic sulfones. According to these workers, this reagent seems to be best adapted to the oxidation of alkyl sulfides below heptyl.

Benzoyl peroxide appears to be a suitable oxidizing agent for the quantitative formation of both aromatic and aliphatic sulfones.[13]

Fichter and Sjöstedt[14] have used electrolytic oxidation to convert certain sulfides to sulfoxides and sulfones.

We have chosen dilute nitric acid as the most suitable reagent for oxidation of the long-chained alkyl sulfides to sulfoxides. For the formation of the corresponding sulfones, either an acetic acid solution of hydrogen peroxide or fuming nitric acid are to be recommended, the former reagent probably being more convenient.

The insecticidal properties of the sulfides and their oxidation products are being investigated.

The melting points and yields of the sulfides, sulfoxides, and sulfones discussed in this work are given in Table I.

TABLE I

MELTING POINTS AND YIELDS OF SULFIDES, SULFOXIDES, AND SULFONES

	SULFIDES		SULFOXIDES		SULFONES	
	m p	Yield %	m p	Yield %	m p	Yield %
n-Dodecyl	40-40.5	81	89-90	95	94.5-95.5	95
n-Tetradecyl ...	49-50	70	95-96	35	99.5-100	90
n-Hexadecyl	57-58	76	97-98	50	100-100.5	85
n-Octadecyl	68-69	75	99-100	92	105 5-106.5	90

The yields herein reported are not necessarily maximum yields. This is particularly true in the case of n-tetradecyl and n-hexadecyl sulfoxides where difficulty was encountered in purifying the substances.

[9] Knoll, J. prakt. Chem., 113, 40 (1926).
[10] Fries and Vogt, Ann., 381, 338 (1911).
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[12] Woods and Travis, J. Am. Chem. Soc., 50, 1226 (1928).
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EXPERIMENTAL PART

PREPARATION OF ETHYL STEARATE[15]

Stearic acid (250 g.) was dissolved in 300 cc. of warm absolute ethyl alcohol, and the resulting solution completely saturated with dry hydrogen chloride. After standing at room temperature for six hours the entire mixture was poured into warm water, and the ester layer separated and washed several times with fresh portions of warm water. The product was taken up in 100 cc. of ether and dried over sodium sulfate. After removal of the ether, the ester distilled very constantly at 190-192° (4 mm.). Yield: 248 g. (90 per cent).

PREPARATION OF n-OCTADECANOL[16]

Sixty-five grams (0.2 mole) of ethyl stearate was placed in a 500 cc. bomb of a Parr hydrogenation machine. Six grams of copper-chromium oxides catalyst[17] was added and the bomb filled with hydrogen (1,640 lbs. pressure at 25° C.). The temperature was raised to 250° and maintained there for four and one-half hours. After cooling, the product was removed, dissolved in hot acetone, and filtered. Fifty-two grams (94 per cent) of n-octadecanol (m. p. 57-58°) crytallized from the cooled filtrate.

PREPARATION OF n-OCTADECYL BROMIDE[18]

A mixture of 38 g. of 42 per cent hydrogen bromide and 13 g. of concentrated sulfuric acid was placed in a 250 ml. 3-necked flask equipped with a mechanical stirrer and reflux condenser. Twenty-seven grams (0.1 mole) of n-octadecanol was gradually added and the reactants refluxed with stirring for six hours. Another addition of 19 g. of 42 per cent hydrogen bromide was made and the refluxing continued for one hour. The reaction mixture was poured into water, the bromide layer separated, washed with cold concentrated sulfuric acid, then with water, and finally with sodium carbonate solution. The oil was taken up with ether, and washed once more and the ether solution dried over sodium sulfate. After removal of the ether (water bath) the bromide distilled at 194-195° (6 mm.). Yield: 30.5 g. (91 per cent). A variation of this procedure has been used by McCorkle.[19]

PREPARATION OF n-OCTADECYL SULFIDE

Five grams (0.22 gram atom) of sodium metal was dissolved in 200 cc. of absolute ethyl alcohol. The solution was divided into two equal portions; the first part was saturated with dry hydrogen sulfide and to this was added the second part of the sodium ethylate solution. A solution of 26.8 g. (0.08 mole) of n-octadecyl bromide in 200 cc. of absolute

[15] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 89. (Unpublished).

[16] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 90. (Unpublished).

[17] Folkers and Connors, J. Am. Chem. Soc., 54, 1138 (1932).

[18] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 96.

[19] McCorkle, Doctoral thesis, No. 481, Library, Iowa State College, 1938, p. 85.

alcohol was then added to the alcoholic sodium sulfide solution. The mixture was refluxed overnight (eight hours) on a steam bath and was then filtered hot. Upon cooling the filtrate, the sulfide crystallized in the form of lustrous plates. These were filtered off and melted in distilled water. After cooling, the solid was removed and melted as before until the water no longer showed any cloudiness in the presence of the melted sulfide. The product was allowed to dry and when recrystallized from acetone weighed 16 g. (75 per cent) and melted at 68-69°.

Anal. Calcd. for $C_{36}H_{74}S$: S, 5.94 per cent. Found: S, 5.93 per cent.

PREPARATION OF n-OCTADECYL SULFOXIDE

(A) One gram (0.00185 mole) of *n*-octadecyl sulfide was dissolved in 100 cc. of hot glacial acetic acid. To this was added 0.25 g . (0.0025 mole) of chromic acid (CrO_3) in ½ cc. of water and 5 cc. of acetic acid. The brownish-green solution that immediately developed changed to a clear green solution. The mixture was kept warm for two hours, cooled, and the crystals filtered off. Upon recrystallization from acetic acid, these melted at 99-100° and weighed 0.92 g. (92 per cent).

Anal. Calcd. for $C_{36}H_{74}SO$: S, 5.77 per cent. Found: S, 5.86 per cent, 5.65 per cent.

(B) One gram of *n*-octadecyl sulfide was added to 100 cc. of dilute nitric acid (50 cc. concentrated nitric acid in 50 cc. of water). The mixture was warmed on a steam bath for thirty minutes, cooled, filtered. and the solid material recrystallized from acetone. The product weighed 0.3 g. (30 per cent) and melted at 99-100°. A mixed melting point with the product of (A) above showed no depression.

PREPARATION OF n-OCTADECYL SULFONE

(A) One gram of *n*-octadecyl sulfide was dissolved in 100 cc. of warm glacial acetic acid. Five cubic centimeters of 30 per cent hydrogen peroxide was added and the solution kept warm for four hours. The solution was cooled and the crystals filtered off. The dried product weighed 0.9 g. (90 per cent) and melted at 105.5-106.5°.

Anal. Calcd. for $C_{36}H_{74}SO_2$: S, 5.61 per cent. Found: S, 5.61 per cent, 5.58 per cent.

(B) One gram of *n*-octadecyl sulfide was added to 50 cc. of fuming nitric acid and the mixture warmed on a steam bath for one hour. The acid mixture was poured into water, filtered, and the solid recrystallized from acetic acid. One-half gram of product (50 per cent yield) was obtained which melted at 105.5-106.5°. A mixed melting point with the sulfone obtained in (A) above was not depressed.

(C) In an effort to make *n*-octadecyl sulfoxide from 1 g. of the sulfide and 0.25 g. of chromic acid in acetic acid, a product was obtained from one run that melted low (92-96°). This was treated once more with 0.25 g. of chromic acid in acetic acid. The product weighed one gram and melted sharply at 105.5-106.5°. A mixed melting point with an authentic specimen of *n*-octadecyl sulfone showed no depression. This is significant

inasmuch as Knoll[20] states that even though a moderate excess of chromic acid is used the sulfoxide will be the product.

REDUCTION OF n-OCTADECYL SULFOXIDE TO n-OCTADECYL SULFIDE

One-half gram of n-octadecyl sulfoxide was dissolved in 100 cc. of hot acetic acid. Zinc dust (2 g.) was added and the mixture refluxed for ten hours. The solution was filtered and allowed to cool. Silvery plates deposited which melted over a wide range (68-85°). The material was redissolved in acetic acid and refluxed with zinc dust for ten hours more. The hot solution was filtered, cooled, the crystals filtered off and washed with water. The dried product melted at 68-70°, and when mixed with pure n-octadecyl sulfide, the melting point was 68-70°. The yield was practically quantitative.

OXIDATION OF n-OCTADECYL SULFOXIDE TO n-OCTADECYL SULFONE

One-half gram of n-octadecyl sulfoxide was dissolved in 100 cc. of acetic acid and 3 cc. of 30 per cent hydrogen peroxide added. The mixture was warmed for two hours, allowed to cool, and the crystals filtered off. After thorough washing and drying, the sulfone was obtained in quantitative yield and melted at 105.5-106.5°. A mixed melting point with pure n-octadecyl sulfone showed no depression.

ATTEMPTED REDUCTION OF n-OCTADECYL SULFONE

One-half gram of n-octadecyl sulfone was dissolved in 80 cc. of hot acetic acid. To this was added 3 g. of zinc dust, and the mixture was refluxed for ten hours. The hot solution was filtered, cooled and the product filtered off. After washing and drying, the sulfone was quantitatively recovered and melted at 105.5-106.5°. A mixed melting point determination with pure n-octadecyl sulfone showed no depression. These results are in agreement with the generally known fact that sulfones are not reduced by the action of zinc in acids, whereas sulfoxides are reduced to sulfides by this treatment.

PREPARATION OF n-HEXADECYL BROMIDE

n-Hexadecyl bromide was prepared by the same procedure as that used for n-octadecyl bromide. Forty-eight and four-tenths grams (0.2 mole) of n-hexadecanol yielded 44 g. (72 per cent) of n-hexadecyl bromide boiling at 178-179° (5 mm.).

PREPARATION OF n-HEXADECYL SULFIDE

In accordance with the procedure as given under n-octadecyl sulfide, 20 g. (0.065 mole) of n-hexadecyl bromide yielded 12 g. (76 per cent) of n-hexadecyl sulfide melting at 57-58°.

Anal. Calcd. for $C_{32}H_{66}S$: S, 6.64 per cent. Found: S, 6.44 per cent.

PREPARATION OF n-HEXADECYL SULFOXIDE

Following the method described for n-octadecyl sulfoxide, 1 g. of n-hexadecyl sulfide upon oxidation in dilute nitric acid gave 0.5 g. (50 per cent) of n-hexadecyl sulfoxide. Some difficulty was encountered

[20] Knoll, *J. prakt. Chem.*, 113, 40 (1926).

when the product was being purified by melting in water. An emulsion was formed that was broken only upon addition of acid. The substance so obtained was filtered off and recrystallized from acetone, m. p. 97-98°.

Anal. Calcd. for $C_{32}H_{66}SO$: S, 6.42 per cent. Found: 6.28 per cent.

PREPARATION OF n-HEXADECYL SULFONE

n-Hexadecyl sulfone was prepared by oxidation of 1 g. of *n*-hexadecyl sulfide with 5 cc. of 30 per cent hydrogen peroxide in 100 cc. of glacial acetic acid. The product obtained in 85 per cent yield (0.85 g.) melted at 100-100.5° after recrystallization from acetic acid.

Anal. Calcd. for $C_{32}H_{66}SO_2$: S, 6.23 per cent. Found: S, 6.21 per cent.

PREPARATION OF n-TETRADECYL BROMIDE

n-Tetradecyl bromide was prepared in the same manner as *n*-octadecyl bromide. One hundred and fifteen grams (0.54 mole) of *n*-tetradecanol gave 111 g. (75 per cent) of *n*-tetradecyl bromide. This substance boiled at 153-154° (6 mm.).

PREPARATION OF n-TETRADECYL SULFIDE

Following the same procedure as given for *n*-octadecyl sulfide, 20 g. (0.072 mole) of *n*-tetradecyl bromide yielded 11 g. (70 per cent) of *n*-tetradecyl sulfide. The product melted at 49-50° after recrystallizing from acetone.

Anal. Calcd. for $C_{28}H_{58}S$: S, 7.51 per cent. Found: S, 7.70 per cent.

PREPARATION OF n-TETRADECYL SULFOXIDE

One gram of *n*-tetradecyl sulfide was oxidized with dilute nitric acid in the same manner as described under *n*-octadecyl sulfoxide. Similar difficulty was encountered as with the purification of *n*-hexadecyl sulfoxide, and only 0.35 g. (35 per cent) of *n*-tetradecyl sulfoxide was recovered. The substance after recrystallization from acetone melted at 95-96°.

Anal. Calcd. for $C_{28}H_{58}SO$: S, 7.25 per cent. Found: S, 7.20 per cent.

PREPARATION OF n-TETRADECYL SULFONE

n-Tetradecyl sulfide (1 g.) was oxidized to *n*-tetradecyl sulfone by 5 cc. of 30 per cent hydrogen peroxide in 100 cc. of acetic acid. Nine-tenths gram (90 per cent) of the sulfone was obtained after recrystallization from acetic acid and melted at 99.5-100°.

Anal. Calcd. for $C_{28}H_{58}SO_2$: S, 6.98 per cent. Found: S, 6.97 per cent.

PREPARATION OF n-DODECYL BROMIDE

The preparation of *n*-dodecyl bromide was similar to that of *n*-octadecyl bromide. It was found advantageous, however, to add a small amount of dilute sulfuric acid to the water used in washing the ether solution of the bromide. The ether solution was dried with sodium sulfate and the solvent removed on a steam bath. The residual oil distilled between 135° and 145° (3 mm.). This was redistilled, and from the 57 g.

(0.3 mole) of n-dodecanol used, 70 g. (90 per cent) of n-dodecyl bromide was obtained boiling at 175-178° (45 mm.).

A similar preparation of n-dodecyl bromide has been given by Kamm and Marvel.[21]

PREPARATION OF n-DODECYL SULFIDE

Preparation of n-dodecyl sulfide was similar to that of n-octadecyl sulfide. Twenty grams (0.08 mole) of n-dodecyl bromide yielded 12 g. (81 per cent) of n-dodecyl sulfide. This product was recrystallized from acetone and melted at 40-40.5°.

Anal. Calcd. for $C_{24}H_{50}S$: S, 8.65 per cent. Found: S, 8.66 per cent.

PREPARATION OF n-DODECYL SULFOXIDE

One gram of n-dodecyl sulfide was oxidized with dilute nitric acid in the same manner as was n-octadecyl sulfide. The product when recrystallized from acetone melted at 89-90° and weighed 0.95 g. (95 per cent).

Anal. Calcd. for $C_{24}H_{50}SO$: S, 8.28 per cent. Found: S, 8.27 per cent.

PREPARATION OF n-DODECYL SULFONE

One gram of n-dodecyl sulfide was oxidized in acetic acid solution with 5 cc. of 30 per cent hydrogen peroxide. The product weighed 0.95 g. (95 per cent) and after recrystallization from acetic acid melted at 94.5-95.5°.

Anal. Calcd. for $C_{24}H_{50}SO_2$: S, 7.95 per cent. Found: S, 7.76 per cent.

SUMMARY

A number of long-chained n-alkyl sulfides, sulfoxides, and sulfones have been prepared.

[21] Gilman, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, New York (1932), p. 27.

HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES.
III. CARBOXYLIC ACID SALTS AND AMIDES OF
n-DODECYLAMINE AND n-OCTADECYLAMINE[1]

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The generally used methods for the formation of substituted amides are indirect in the sense that the amine does not react directly with an acid itself, but rather with a derivative of an acid such as an acid halide, an acid anhydride, or an ester. Conversion of the acid into these derivatives is time-consuming, and the yields are not always satisfactory. It seems that the more direct method of forming the amine salt of the carboxylic acid and subsequent dehydration of this to the amide is a procedure that has been overlooked to a great extent and probably can be applied advantageously in the preparation of a large number of substituted amides. The method is not a new one, but current references to its application appear to be limited almost entirely to the patent literature.

The first preparation of a substituted amide by pyrolysis of a substituted ammonium salt appears to be that of Wurtz[2] in 1850. Following the method used twenty years earlier by Dumas[3] for the conversion of ammonium oxalate to oxamide, Wurtz distilled dimethylammonium oxalate and obtained dimethyl oxamide. The method was extended to monobasic aliphatic acids by Linneman[4] who evaporated aqueous solutions of methyl-, ethyl- and diethylammonium formates and distilled the resulting syrups to obtain the corresponding amides. The isolation of the intermediate ammonium salt was eliminated by Franchimont and Klobbie[5] who heated n-heptylic acid and several low-molecular-weight amines directly in sealed tubes at about the boiling point of the acid and obtained the desired amides. Tafel and Stern[6] carried the direct condensation technique over to the field of dibasic acids. By heating isopropylamine and succinic acid in a sealed tube they were able to obtain isopropyl succinimide in 75 per cent yield.

Decomposition of acetic acid salts of a number of lower primary and secondary amines was quantitatively studied by Musselius.[7] The salts were heated in sealed tubes at 215° (in nitrobenzene vapor) for thirty minutes, and the unchanged salt was determined by titration with base.

[1] Paper II: *Iowa State Coll. J. of Sci.*, 15, 215 (1941).
[2] Wurtz, *Ann. chim.*, 30, 464 (1850).
[3] Dumas, *Ann. chim. phys.*, 44, 129 (1830).
[4] Linneman, *Proc. Viennese Academy*, 60, 44 (1870); [*Chem. Zentr.*, 41, 138 (1870)].
[5] Franchimont and Klobbie, *Rec. trav. chim.*, 6, 247 (1887).
[6] Tafel and Stern, *Ber.*, 33, 2232 (1900).
[7] Musselius, *J. Russ. Phys. Chem. Soc.*, 32, 29 (1900). [*Chem. Zentr.*, 71, I, 1071 (1900)].

He found that the yield of amide increased with the molecular weight of the amine.

Verley[8] prepared dimethyl formamide and dimethyl acetamide by distilling an alkali salt of the acid and dimethylamine hydrochloride. Reid and co-workers[9] prepared the dimethyl amides of the normal fatty acids from formic to heptoic by passing dimethylamine through the acids at temperatures ranging from 95° to 160° for varying lengths of time, the conditions depending upon the acid used. Phosphorus pentoxide has been used as a dehydrating agent in the formation of certain N-substituted amides.[10]

Several propionamides have been prepared by Bowen and Smith[11] by refluxing amines with propionic acid for several hours. A U. S. patent[12] refers to the production of alkyl substituted amides by heating aliphatic acids and aliphatic amines in the presence of a liquid immiscible with water. The constant boiling mixture is distilled off, removing the water formed in the reaction.

A number of amides from carboxylic acids and n-dodecylamine and n-octadecylamine has been prepared. Harber[13] described the o- and p-chlorobenzamides, o- and m-toluamides, oleamides, elaidamides and anisamides of these amines. N,N'-Di-n-octadecyl malonamide and N-n-dodecyl and N-n-octadecyl phthalimide were also described by the same investigator. A number of the amides reported in Tables I and II have also recently been prepared by Harber [13] who used a modification of the method described in the Experimental Part. The modification consisted of eliminating the step involving the isolation of the intermediate salts. Mixed melting point determinations indicated that the products of either method were identical. N-n-Dodecyl lauramide has been prepared from a mixture of lauramide and lauric acid by heating with hydrogen at elevated temperature and pressure in the presence of a catalyst.[14] Adam and Dyer[15] prepared N-n-octadecyl acetamide and N-n-hexadecyl acetamide by warming the corresponding amines with acetic anhydride. N-n-Dodecyl acetamide is described in the patent literature as having been prepared by heating lauronitrile and acetamide with hydrogen under pressure in the presence of nickel.[16] N-n-Dodecyl benzamide, N-n-dodecyl phenylacetamide and N,N'-di-n-dodecyl oxamide were prepared by Grünfeld[17] from n-dodecylamine and the corresponding ethyl esters. N-n-Octadecylbenzamide has been previously prepared by the action of benzoyl

[8] Verley, *Bul. soc. chim.*, [3] 9, 691 (1893).

[9] Mitchell and Reid, *J. Am. Chem. Soc.*, 53, 1879 (1931); Ruhoff and Reid, *ibid.*, 59, 401 (1937).

[10] German Patent 653,873 [*C. A.*, 32, 2956 (1938)]

[11] Bowen and Smith, *J. Am. Chem. Soc.*, 62, 3522 (1940).

[12] U. S. Patent 1,954,433 [*C. A.*, 28, 3741 (1931)].

[13] Harber. Doctoral Thesis, No. 590, Iowa State College (1940).

[14] German Patent 667,627 [*C. A.*, 33, 2906 (1939)].

[15] Adam and Dyer, *J. Chem. Soc.*, 127, 73 (1925).

[16] British Patent 458,454 [*C. A.*, 31, 3501 (1937)]; German Patent 650,664 [*ibid.*, 32, 953 (1938)].

[17] Grünfeld, *Ann. chim.*, [10] 20, 366 (1933).

chloride on n-octadecylamine.[18, 20] Hoyt [19] prepared N-n-octadecyl stearamide from stearyl chloride and n-octadecylamine. Chloroacetic amides of n-dodecyl-, n-tetradecyl- and n-octadecylamine are mentioned in the patent literature,[21] but the physical constants and methods of preparation are not given.

A number of amides from long-chained amines other than n-dodecylamine and n-octadecylamine have been reported. Turpin[22] prepared N-n-heptadecyl benzamide from n-heptadecylamine and benzoyl chloride. Jeffreys[23] used the same method to form N-n-pentadecyl benzamide from n-pentadecylamine. The acetyl derivatives of these same amines as well as that of n-undecylamine have been described by Naegeli and co-workers.[24] These latter investigators also reported the formation of N-n-heptadecyl stearamide and N-n-pentadecyl palmitamide.

A number of substituted amides from long-chained acids and various amines are reported in the literature. de'Conno[25] mixed equimolar amounts of higher fatty acids and aromatic amines and heated these in evacuated, sealed tubes at 230° for five hours. The yields of amides were good. The p-nitroanilides and 2-nitro-p-toluidides of lauric, myristic, palmitic and stearic acids were prepared by Gilman and Ford[26] by heating the amines with the corresponding acid chlorides. The p-xenylamides of the same acids were prepared by these workers by heating equimolar quantities of the acids and p-xenylamine in sealed tubes for five hours at 135-140°. Robertson [27] prepared the o- and p-toluidides, β-naphthylamides, p-bromoanilides, o-bromo-p-toluidides, α-bromo-β-naphthylamides, and 2,4,6-tribromoanilides of a large number of normal fatty acids and made an interesting study of their melting points. He plotted the melting points of each series of compounds against the number of carbons in the acid radical and noted that the odd and even members fell on two distinct curves, which, although not superimposable, fall and rise in the same manner at corresponding places. A minimum in the curves appeared generally at about C_4. We find that similar results are obtained when one plots the melting points of the even members of the normal fatty acid salts or of the corresponding amides of n-octadecylamine against the number of carbon atoms in the acid radicals. The minimum occurs at six carbons in the case of the salts and at four in the case of the amides. An insufficient number of odd-chained members of these series was prepared to permit a similar comparison.

[18] Hoyt, Doctoral Thesis No. 560, Iowa State College (1940), p. 78.

[19] Ref. 18, p. 83.

[20] Shinozaki and Kubo, J. Agr. Chem. Soc. Japan., 13, 1 (1937); [C. A. 31, 3002 (1937)].

[21] French Patent 735,647 [Chem. Zentr., 104, 1224 (1933)].

[22] Turpin, Ber., 21, 2486 (1888).

[23] Jeffreys, Am. Chem. J., 22, 22 (1899).

[24] Naegeli, Grüntuch-Jacobsen and Lendorff, Helv. Chim. Acta, 12, 227 (1929).

[25] de'Conno, Gazz. chim. ital., 47, I, 93 (1917); [C. A., 12, 1172 (1918)].

[26] Gilman and Ford, Iowa State Coll. J. Sci., 13, 135 (1939).

[27] Robertson, J. Chem. Soc., 115, 1210 (1919).

TABLE I

Salts and Amides of n-Octadecylamine

Acid	Salt					Amide				
	M.P. °C	Mixed M.P. °C	Pctg Yield	Anal. Pctg. N Calcd.	Found	M.P. °C	Mixed M.P. °C	Pctg Yield	Anal. Pctg. N Calcd.	Found
Formic	78.5-79.5		87	4.44	4.44	68-68.5	67-76	95	4.70	4.50
Acetic	84.5-85		87	4.27	4.28	78-78.5§	74-77	90	4.50	4.45
Propionic	78.5-79*		82	4.08	3.92	77-77.5*	74-77	85	4.31	4.26
n-Butyric	71-71.5		81	3.98	3.79	76-76.5	75-76	90	4.14	4.12
n-Valeric	60-61		70	3.78	3.57	76-76.5	75-77	90	3.98	4.05
Caproic	55-56	54-57	80	3.64	3.73	78-78.5	73-76	90	3.81	3.91
Caprylic	57.5-58		94	3.38	3.23	79-79.5	77-80	85	3.65	3.38
Capric	62-62.5		84	3.17	3.28	83-83.5	81-84	91	3.30	3.06
Lauric	68-69		88	2.98	3.08	87.5-88||	85-87	85	3.10	2.98
Myristic	78-78.5		91	2.82	3.06	89-89.5||	88-90	85	2.95	3.10
Palmitic	85-85.5	84-88	95	2.67	2.90	91.5-92||	91-94	90	2.76	2.96
Stearic	89.5-90.5		93	2.53	2.30	95.5-96||¶		92	2.62	2.72

Benzoic	65 -66	95	3.59	3.45	85.5-86‖**††	95		
Anthranilic	92.5-93.5	92	6.90	6.72	‡‡			
α-Furoic	91 -92	95	3.68	3.56	79.5-80.5§	93	3.86	3.91
Cinnamic	80.5-81.5	93	3.36	3.27	90-91	93	3.51	3.50
Salicylic	73.5-74†	97	3.44	3.32	74.5-75.5†‖‖	25	3.61	3.82
Phenylacetic	85 -85.5	90	3.51	3.42	94.5-95	75	3.62	3.60
Oxalic	203 -205‡	64	4.45	4.40	120-121	61	4.73	4.64
α-Naphthoic	109 -110	92	3.17	3.14	89.5-90¶¶	85	3.32	3.48
2-Dibenzofuran-carboxylic	88 -89‡	71	2.91	3.04	118-118.5	85	3.02	3.04

* A mixture of N-n-octadecylammonium propionate and N-n-octadecyl propionamide melted at 74-77°.
† A mixture of N-n-octadecylammonium salicylate and N-n-octadecyl salicylamide melted at 70-73°
‡ Ethyl alcohol was the solvent used in preparing this salt
§ Prepared previously (ref. 15).
‖ Also prepared directly from acid and amine without isolation of salt (ref 13).
¶ Prepared previously from acid chloride, Hoyt gives m.p. of 96-97 (ref. 19)
** Prepared previously from acid chloride, Hoyt gives m.p. of 85-87 (ref. 18).
†† Also prepared from benzoyl chloride and amine; m.p. and mixed m.p. 85.5-86°
‡‡ No pure product was obtained upon attempted dehydration of salt
§§ Also prepared from furoyl chloride and amine; m.p. and mixed m p., 79.5-80.5°,
‖‖ Heating of N-n-octadecylammonium salicylate effected partial decomposition and the odor of phenol was detected.
¶¶ Also prepared from α-naphthoyl chloride and amine; m p. and mixed m.p. 89-90°.

TABLE II
Salts and Amides of n-Dodecylamine

Acid	Salt M.P. °C	Salt Mixed M.P. °C	Salt Pctg. Yield	Salt Anal Pctg. N Calcd.	Salt Anal Pctg. N Found	Amide M.P. °C	Amide Mixed M.P. °C	Amide Pctg. Yield	Amide Anal Pctg. N Calcd.	Amide Anal Pctg. N Found
Acetic	67-68		90	5.71	5.54	53-54‡	50-53	90	6.15	5.85
n-Propionic	56-57*		64	5.41	5 25	53-53.5*		77	5.80	5.60
Lauric	72-73		91	3.63	3.59	77-77.5§	77-81	90		
Myristic	72.5-73	67-68	93	3.39	3.38	84-85§		85		
Palmitic	72-73	68-70	93	3.17	2.99	82.5-83§	82-84	85		
Stearic	69-70†	69-71	92	2.93	2.93	85-85.5§	82-84	85		
α-Furoic	72.5-73		91	4.73	4.48	57-58‖		88	5.03	5.28
Phenylacetic	68.5-69.5		65	4.36	4.06	79-79.5¶		80	4.62	4.59
α-Naphthoic	114-115		73	3.97	3.73	71-72		20	4.13	3.96
Chloroacetic	65-66		75	5.00	4.86	(**)				
2-Dibenzofuran-carboxylic	87.5-88.5		75	3.52	3.40	112-113		80	3.70	3.96
Cinnamic	53.5-55		75	4.20	4.23	74-74.5		80	4.45	4.78

*Mixed m.p of N-n-dodecylammonium propionate and N-n-dodecyl propionamide was 48-54°.

†Mixed m.p. of N-n-dodecylammonium stearate and stearic acid was 59-67°.

‡Prepared previously (ref. 16 and ref. 13, p. 100).

§Also prepared directly from acid and amine without isolation of salt The analyses are reported elsewhere (ref. 13).

‖Also prepared from furoyl chloride and amine; m.p. and mixed m p 57-58°.

¶Prepared previously (ref 17).

**Preparation is in progress.

A long series of fatty acid diamides of ethylene diamine has been prepared by Tucker,[28] who heated the ethyl esters of the fatty acids (from C_2 to C_{17}, except C_9) with ethylene diamine. Details for the preparation of hexamethylene distearamide and hexamethylene dilauramide are given in a U. S. Patent.[29] Such compounds may be used as high-melting waxes, plasticizers, or as "slip-promoting agents" to prevent sheet materials from sticking together. We have prepared the salt of oxalic acid and octadecylamine and found that this substance is also very high melting (203-205°) and exhibits the "feel" of talc.

In the preparation of N-substituted amides of long-chained amines we have found that the direct formation from the acid and amine takes place very smoothly and in very good yields. The intermediate salts are usually readily isolated and generally lend themselves to ready purification from excess of either of the starting materials. Best results are obtained, however, if the acid and amine are mixed in exact molecular proportions. The isolation of the salt is not essential, and by careful measuring of molecular quantities of both acid and amine Harber[30] has shown that good yields of pure amides may be obtained by simply heating the reactants together in an atmosphere of nitrogen. The inert atmosphere is used to reduce darkening due to air oxidation at the temperature necessary for dehydration of the amine salt.

The long-chained amine salts were readily prepared by warming the amine with a carboxylic acid in a suitable solvent. Petroleum ether (b. p., 60-68°) was the solvent most generally used; however, ethyl alcohol served very well in the case of less soluble salts. No ester formation was observed. The amine salts are usually considerably less soluble in cold petroleum ether than the long-chained amines or fatty acids and thus are easily separated from these impurities. Certain carboxylic acids, however, are quite insoluble in petroleum ether and dissolve only upon addition of the amine to the petroleum ether mixture. The acid dissolves only to the extent to which it reacts to form the salt, and hence excess acid may be filtered from the hot solution. Upon cooling, the pure salt crystallizes from the filtrate.

EXPERIMENTAL PART

PREPARATION OF AMINE SALTS

The following procedure illustrates the method that has been found satisfactory for a large number of amine salts:

To a solution of 2.69 g. (0.01 mole) of *n*-octadecylamine in 100 cc. of petroleum ether (b. p., 60-68°) was added 2.84 g. (0.01 mole) of stearic acid. The mixture was warmed until all had passed into solution. The warm mixture was filtered and the filtrate allowed to cool slowly until the salt crystallized out. The product was filtered and dried on a Büchner fun-

[28] Tucker, *J. Am. Chem. Soc.*, **57**, 1989 (1935).
[29] U. S. Patent 2,132,388 [*C. A.*, **33**, 174 (1939)].
[30] Harber, Doctoral Thesis No. 590, Iowa State College (1940).

nel. The yield of N-*n*-octadecylammonium stearate was 5.15 g. (93 per cent). Successive recrystallizations from petroleum ether and alcohol failed to raise the melting point (89.5-90.5°). The salt was analyzed by the micro Kjeldahl method.

PYROLYSIS OF AMINE SALTS TO AMIDES

Conversion of the amine salts to amides could usually be effected by simply heating the salt in a nitrogen atmosphere between 225-250° for from fifteen to thirty minutes. Water was smoothly evolved under these conditions and very little discoloration of the product was observed. The amide was purified by recrystallization from petroleum ether or alcohol and in most cases was obtained in practically theoretical yield. A typical procedure is illustrated in the preparation of N-*n*-octadecyl stearamide:

N-*n*-Octadecylammonium stearate (2 g.) was heated in a nitrogen atmosphere on a Wood's metal bath between 225-250° for fifteen minutes. The cooled product weighed 1.95 g. (98 per cent) and melted constantly at 94.5-95° after two recrystallizations from petroleum ether.

The melting points, yields, and analyses of the salts and amides of *n*-octadecylamine and *n*-dodecylamine are given in Tables I and II, respectively. Mixed melting points of contiguous members whose melting points were within 5° are also given. As the depressions are generally slight, this method is of questionable value in differentiating these substances. The limitations of mixed melting point determinations in differentiating successive members in a series of long-chained derivatives has been noted previously by Gilman and Ford,[31] by McCorkle,[32] and others.

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SUMMARY

The preparation of a number of aliphatic and aromatic carboxylic acid salts and amides of *n*-dodecylamine and *n*-octadecylamine has been described. The method of amide formation is more direct than those generally used inasmuch as the carboxylic acid itself rather than a derivative of the acid reacts directly with the amine.

The melting point curves for a series of salts and a series of amides of *n*-octadecylamine and normal fatty acids show a minimum point at C_6 in the case of the salts and at C_4 in the case of the amides.

[31] Gilman and Ford, *Iowa State Coll. J. Sci.*, 13, 135 (1939).
[32] McCorkle, Doctoral Thesis No. 481, Iowa State College (1938).

LASIUS (ACANTHOMYOPS) PLUMOPILOSUS, A NEW ANT WITH PLUMOSE HAIRS FROM IOWA

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Lasius (Acanthomyops) plumopilosus sp. nov.

FEMALE

Length, 4.6 mm.

Head excluding the mandibles very nearly as broad (.97 mm.) as long (1.00 mm.), with very feebly excised posterior border and slightly convex sides. Eyes convex, hairy. Mandibles 6-dentate. Head without a frontal furrow. Scapes nearly reaching the posterior corners of the head, gradually thickened distally. Funiculi less incrassated than in *claviger*, joints 2-10 broader than long, the penultimate joints very little broader than long. Thorax 1.70 mm. long, .87 mm. wide (across the wing insertions); mesonotum and scutellum forming a nearly flat surface above. Epinotum with a very short base and steep declivity. Petiole small, its superior border faintly notched and blunter than in *L. (A.) claviger* and *interjectus*, the scale a little less than two-thirds as high as the epinotum. Gaster long, the sides subparallel. Head and body in profile formed about the same as in *claviger*. Tibiae and femora flattened, fore femora about 3 times, middle and hind femora about 4 times, as long as broad. Wings 5.4 mm. long.

Whole body strongly shining with pilosity and pubescence arising from punctures. Mandibles finely striate.

Pilosity extraordinary, nearly all of the erect hairs plumose or at least branching at the tip. Plumose hairs best developed on the head, thorax, petiole, and mid-dorsal region of the first gastric segment. Pinnules of the hairs in no case covering more than the distal half of a hair, in many cases not covering more than the distal one-fourth or one-sixth, and in many cases on the gaster merely 3, 4, or 5-branched at the tip. Number of pinnules on well-developed plumose hairs about 8-15. Hairs present on the clypeus, on the front in a fringe pointing inwardly, on the vertex, occiput, gula, pronotum, prosternum, on the mesonotum in a fringe pointing inwardly, on the scutellum, mesepisternum, mesosternum, metanotum, epinotum, and petiole; very numerous on the gaster including the ventral side of the first segment (bases of contiguous hairs on the gaster about .04-.10 mm. apart). Succeeding gastric segments on the ventral side with a row or two of hairs along their posterior margins. Also

present although much smaller and fewer on the coxae, trochanters, and fore femora. Length of hairs about .16-.20 mm.; of well-developed pinnules, about .03-.04 mm. Pubescence rather sparse on most parts of the body, denser on the legs, antennae, ventral surface of the pedicel, and base of gaster. Second and succeeding gastric segments with bands of very fine, short pubescence on the anterior margins, changing posteriorly into sparser, longer pubescence. Pubescence on the genae and gula very long, strong, sparsely set, and subappressed.

Color much darker brown than in the females of *interjectus* and *latipes*, the head and thorax appearing brownish black to the naked eye; not as dark, however, as in some specimens of *claviger*.

WORKER

Length, 3.2 mm.

Head, excluding the mandibles very nearly as broad (.93 mm.) as long (.94 mm.), with a very slightly excised posterior border and convex sides; widest across the middle of the head. Mandibles 6-dentate. Eyes hairy, slightly convex, with about 45 ommatidia. Scapes not quite reaching the posterior corners of the head. Funiculi incrassated, joints 2-9 at least slightly broader than long, joint 10 about as broad as long. Thorax with the shape of *claviger;* 1.16 mm. long and .58 mm. wide (across the pronotum); meso-epinotal suture relatively shallow. Petiole not quite as high as the epinotum, the superior border notched and about as blunt as in *claviger;* having about the same shape as in *claviger* when seen from behind; anterior surface convex, posterior flat. Femora and tibiae flattened, fore femora about 3 times, middle and hind femora about 4 times, as long as broad.

Whole body very shining. Mandibles finely striate.

Many of the erect hairs plumose or branching at the tip, although not as much as on the female. Plumose hairs best developed in a row on the occiput, on the pronotum, mesonotum, epinotum, and mid-dorsal region of the first gastric segment. Hairs present whether plumose or not on the clypeus, front, occiput, gula, pronotum, mesonotum, epinotum, petiole, coxae, and fore femora; numerous on the gaster including the ventral side of the first segment. Succeeding segments on the ventral side with a row or two of hairs along their posterior margins. Length of hairs about .10-.15 mm. Pubescence sparse, denser on the legs, coxae, and antennae. Second and succeeding gastric segments with bands along the anterior margins of very fine, short pubescence. Pubescence on the genae and gula very long and strong.

Color sordid yellow, mandibles and funiculi more brownish.

MALE

Length, 3.2 mm.

Head excluding the mandibles little broader (.80 mm.) than long (.75 mm.), broader behind than in front, with rather convex posterior border and very slightly convex sides. Eyes hairy, very convex, situated

on the sides halfway between the corners of the head. Mandibles with 6
teeth, but counting from the basal border, only numbers 4, 5, and 6 on
the right mandible and numbers 3, 5, and 6 on the left mandible very
distinct. Frontal furrow distinct. Antennae long and slender, the scapes
surpassing the posterior corners of the head. Funiculi with first joints
swollen, other joints cylindrical, joints 2-11 very gradually increasing in
length. Thorax 1.42 mm. long, through the wing insertions less broad
(.74 mm.) than the head. Declivity of the epinotum more sloping than in
the female. Petiole with a very sharp border, its anterior surface slightly
concave and its posterior surface slightly convex. Wings 4.2 mm. long,
discal cell absent.

Less shining than the worker and female; the head and thorax finely
punctate and subopaque.
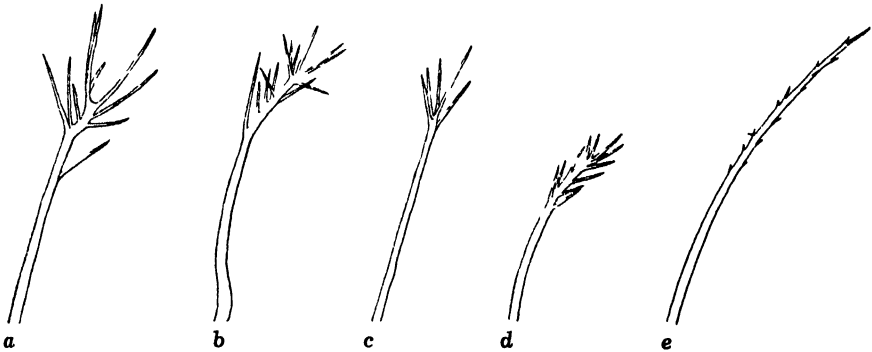
FIG. 1. *L. (A.) plumopilosus* hairs: *a* and *b,* from mesonotum of female; *c,* from
abdomen of female; *d,* from pronotum of worker. *L.(A.) claviger* hair: *e,* from thorax
of female.

Hairs sparser, and more slender than in the worker and female, a
little longer (about .11-.16 mm.) than in the worker; not plumose gener-
ally, although several hairs on the clypeus, vertex, occiput, mesonotum,
and scutellum branching at the tip. Pubescence more sparse than in the
female and not as varied in size.

Color very dark brown appearing black to the naked eye. Head and
mesonotum very nearly black. Funiculi and legs infuscated yellow.

VARIATION IN TYPE MATERIAL

Both females and workers vary somewhat in the plumosity of the
hairs, in the arrangement of the hairs, and in the location of the most
plumose ones. In general, however, they are as described. There is little
difference in size in either females or workers. Several of the paratype
females, however, have the gaster wider and larger than in the holotype.
The discal cell of the fore wing is very variable, being either present,
incomplete, or absent. The holotype has the discal cell present on the
left wing, absent on the right. The shape of the petiole varies also; one
female seems to be without even a slight notch on the superior border,
which is evenly rounded above.

Described from a series of 8 alate females, 6 workers, and 1 male taken from a colony nesting under a hillside rock at Backbone State Park, Iowa, September 13, 1940.

Holotype female, 1 paratype female, 2 paratype workers, and the paratype male are to be deposited in the National Museum, Washington, D. C.; 3 paratype females and 2 paratype workers are to be deposited in the Iowa State College collection; and 3 paratype females and 2 paratype workers are to remain in the author's collection.

This species appears to be closely related to L. (A.) claviger from which it can be separated very definitely by the following points: (1) the highly specialized plumose hairs of the female and worker, (2) the smaller size of the worker and the decidedly smaller size of the female and male, (3) the 6-dentate mandibles of the male, and (4) the much less incrassated funiculi of the female, the penultimate joints being only about 1.1 times as broad as long, whereas in the females of claviger that the writer has measured the penultimate joints are 1.3 to 1.4 times as broad as long. Other differences include: (1) the very abundant hairs on the gaster of the female, (2) the proportionately shorter hairs on the female and worker, (3) the more highly specialized pubescence on the female and worker, (4) the less flattened femora and tibiae of the female, and (5) the proportionately more slender body of the female and male.

Two other species of the subgenus Acanthomyops, L. (A.) interjectus coloradensis Wheeler and L. (A.) interjectus mexicanus Wheeler, also have very small females 5 mm. long or less, very small workers about 3 mm. long, relatively short antennae, and males with dentate mandibles. From Wheeler's descriptions of coloradensis and mexicanus, plumopilosus can probably be distinguished by at least the following points: (1) the highly specialized plumose hairs on the female and worker, (2) the edentate basal border of the mandibles in the female and worker, (3) the shorter antennae, the scapes not reaching the hind corners of the head, (4) the much more numerous hairs, especially on the gaster of the female, than on mexicanus, at least, (5) the greatly varied pubescence which is also decidedly less profuse than on mexicanus, and (6) the more flattened legs of the female and worker. Future comparisons of these two forms and especially of coloradensis with plumopilosus will perhaps throw more light on the latter's affinities.

As far as the writer has been able to ascertain, this is the first recorded instance of plumose hairs in the Formicidae. At least this type of pilosity is very rare among ants. However, examination under the highest power of the binoculars shows some females of L. (A.) interjectus, claviger, and latipes to have hairs with a very poorly developed type of plumosity consisting of short, very tiny, rather suberect pinnules which tend to be scattered along the length of the hair rather than collected distally. Short, very tiny pinnules can sometimes be seen on the hairs of the workers of interjectus, claviger, and latipes, also. The author has not had the opportunity of examining specimens of claviger subsp. subglaber, occidentalis,

murphyi, or any of the subspecies of *interjectus* and so cannot make any statements regarding them.

Many of the North American *Lasii* of the subgenera *Acanthomyops* and *Chthonolasius* are considered to be temporary social parasites of the varieties of *L. niger.* The very small size and extraordinary pilosity of the females of *plumopilosus* seem to suggest, however, that this species may be a more highly specialized parasite than other species of this genus.

CONCERNING SOME HALOBATINIDS FROM THE WESTERN HEMISPHERE (HEMIP. GERRIDAE)

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The present paper contains the descriptions of two new Halobatinae from Panama and Brazil and notes on some other gerrids from the Western Hemisphere. The types are in the collection of the authors.

HALOBATOPSIS PLATENSIS (Berg)

Halobates platensis Berg, Anales Soc. Cient. Arg., 8, 1879, p. 24 (Hemip. Arg., p. 183).
Halobatopsis platensis Drake and Harris, Notas Museo La Plata, III, 1938, p. 20, fig. 1.

This species shows considerable variation in color. It is known from Argentina and Brazil. Heretofore, only the apterous form was known.

Winged form: Markings on head same as in apterous. Pronotum large, longer than wide, subtriangularly produced behind, convex above, the apex broadly rounded; largely black, with an irregular discal spot on anterior lobe and two large patches on hind lobe testaceous to brownish testaceous. The somewhat rectangular spot on the front of the pronotum connected at its outer hind angles to the lateral pale stripe by a fine, sometimes incomplete, curved pale line. Hemelytra black, opaque, the veins darker and hairy. In four of the specimens the wings are broken off at the transverse suture.

Morphotype, Belo Horizonte, Minas Geraes, Brazil, Oscar Monte, in authors' collection.

Halobatopsis delectus, sp. nov.

Apterous form: Similar to *H. spiniventris* Drake and Harris, but readily distinguishable by the raised knob within near the basal fourth of the fore femora, the sinuate and moderately swollen fore tibiae near the middle, and the distinctly sharper, narrower and more deeply excavated notch on the hind margin of the last venter of the male. The posteriorly projecting spine in the notch of the last venter of male testaceous, moderately prominent, projecting a little beyond hind margin of venter; first genital segment testaceous, longitudinally ridged along median line; the last segment embrowned distally, there clothed with brown hairs. Last segment of venter long, a little longer than two preceding segments, very broadly, roundly excavated behind. Fore legs brownish black, the femora curved, with a broad, testaceous stripe along the greater portion of outer surface. Middle and hind legs brownish black. First genital above notched behind, dark brown distally.

In both sexes the second antennal segment a little longer than in *spiniventris,* connexiva of female with tufts of dark hairs as in *spiniventris.* Last venter in female as long as three preceding. The fore legs not modified as in male. Color and markings very similar to *spiniventris* (Drake and Harris, Rev. Ent., VII, 4, 1934, p. 358, fig. 2c). Fore legs of female not modified, the hind femora with hairs within as in *spiniventris.*

Length, 4.00-4.60 mm.; width, 1.75-2.00 mm.

Holotype (male), *allotype* (female), and 3 *paratypes,* Belo Horizonte, Minas Geraes, Brazil, collected by Oscar Monte.

This is the fourth member of the genus and the only one having modified front femora and tibiae in the male. The peculiar spine of the last venter of male is dark brown in *spiniventris* and testaceous in *delectus.*

TELMATOMETRA WHITEI Bergroth

Telmatometra whitei Bergroth, Ohio Nat., VIII, 1908, p. 374; Drake and Harris, Rev. Ent., VII, 1937, p. 360, fig. 2a.

Originally described from 5 winged males and females from Escuintla, Guatemala. Many apterous specimens and 2 winged examples are at hand from Barro Colorado Island, Canal Zone, Gatum and Panama City, Panama, collected by C. J. Drake; Mayaguez, Puerto Rico, H. D. Tate; Moralies, Guatemala, J. J. White; and Punta Gorda, British Honduras, J. J. White. The color markings exhibit considerable variation in a series of specimens taken at one station in the wide reaches of a stream near old Panama City. The black lateral stripes of the pronotum vary much in width. The median stripe may be entirely wanting or may vary from a short basal stripe to a complete longitudinal, median stripe, which also varies considerably in width. The forms with a total median line agree perfectly with Esaki's *T. ujhelyii.* Specimens of typical *whitei* and *ujhelyii* were taken by C. J. Drake in the same school in wide reaches of a stream at old Panama City and in a quiet pool of a small stream at Barro Colorado Island, Canal Zone, Panama.

TELMATOMETRA WHITEI UJHELYII Esaki

Telmatometra ujhelyii Esaki, Ann. Mus. Nat. Hung., XXIII, 1926, p. 133, fig. 4.

This form is treated here as a color variety of *whitei* Berg. There are no antennal, genital or other structural differences which will distinguish the two forms. Winged specimens of *whitei* agree perfectly with Esaki's description and figure. The extreme color differences of apterous forms seem to warrant the retaining of *ujhelyii* as a color variety.

TELMATOMETRA ROZEBOOMI Drake and Harris

Telmatometra rozeboomi Drake and Harris, Rev. de Entomologia, VII, 1937, p. 358, fig. 2b.

One male, taken in the wide reaches of stream near old Panama City,

Pan., Feb. 10, 1939, C. J. Drake. This species is considerably smaller, very differently colored and has also a great difference in genital character from *whitei*. It is smaller and differs markedly in external genital structure from *panamensis*, n. sp.

Telmatometra panamensis, sp. nov.

Apterous form: Moderately elongate, yellowish brown, with prominent black-fuscous markings. Head with sides and lateral margins above (widening posteriorly) fuscous-black, the broad central portion (narrowing posteriorly) and a very narrow margin behind testaceous. Eyes blackish. Rostrum long, extending about one-third of its length beyond mesosternum, testaceous, the distal third blackish. Antennae long, slender, brownish black; antennal proportions—I, 16; II, 9; III, 22; IV, 20. Pronotum nearly twice as long as broad, broadly impressed on the disc, testaceous above, the sides and a very narrow border in front and behind black-fuscous; mesonotum long, dorsal surface and a wide enclosed strip on each side testaceous, the rest, including a narrow front margin and a short, median, basal streak black-fuscous; mesonotum with a large spot on each side testaceous, the median black stripe becoming wider posteriorly. Abdomen above testaceous, the first two segments, sutures between segments, and narrow outer margins of connexiva black-fuscous. Body beneath testaceous, anterior legs mostly dark brown, the femora above with a broad, longitudinal, testaceous stripe; tibiae also with a pale stripe along dorsal side. Middle and hind legs dark brown, the femora beneath testaceous.

Winged form: Pronotum very large, long, convex above, nearly triangular behind, rounded at apex, testaceous, the sides very broadly margined with blackish fuscous, narrow in front, a very large broad, triangular spot on the disc extending to the hind margin as a median line dark fuscous. Hemelytra black-fuscous, the veins hairy, darker and prominent, broken off on the five examples at the suture.

Male: Last venter long, as long as three preceding, the hind margin broadly concavely rounded; first genital segment beneath long, flat, hairy, sharply triangularly notched behind; the second segment short, rounded behind.

Female: Broader than male. Last venter very long, longer than three preceding segments, very broadly and deeply excavated behind; first genital segment long, broad, flattened beneath, the terminal segment very short.

Length, 3.95 mm.; width, 1.25-1.55 mm.

Holotype (male), *allotype* (female), and winged *paratypes*, Canal Zone, Panama, Feb. 10, 1939, C. J. Drake.

This species may be easily distinguished from its congeners by the color markings, the last venter, and male genital segments.

CHARMATOMETRA BAKERI (Kirkaldy)

Brachymetra bakeri Kirkaldy, Entom. XXXI, 1898, p. 101.
Charmatometra bakeri Kirkaldy, Ann. Ent. Soc. Belg., XLIII, 1899, p. 509.

Recorded in the literature from Ecuador and Colombia. The writers also have several specimens from Venezuela. It is the largest halobatid recorded from the Western Hemisphere. The macropterous form is unknown.

CYLINDROSTHETHUS PALMARIS Drake and Harris

Cylindrosthethus palmaris Drake and Harris, Ann. Carn. Mus., XXIII, 1934, p. 222.

Many apterous specimens, Trinidad, B. W. I., Oct. 27, 1938, C. J. Drake. This species was found in considerable numbers in the wide reaches of small streams at many different points in the island. It is a very active species and moves rapidly over the surface of the water when disturbed. This species and the following belong to the subfamily Gerrinae.

CYLINDROSTHETHUS LINEARIS Erichson

Cylindrosthethus linearis Erichson, in Schmburgh's Fauna Brit. Guiana, III, 1848, 18, p. 614; Drake and Harris, Ann. Carnegie Mus., XXIII, 1934, p. 220.

One male, Trinidad, Bolivia, Collection of M. S. Pennington. Known heretofore only from British Guiana and Rio de Janeiro, Brazil.

SOME NEW SPECIES OF MIRIDAE (HEMIPTERA) FROM CHINA

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The following descriptions of eleven new species and one new variety are based on a small collection of Chinese mirids in the possession of the United States National Museum and some specimens kindly sent the writer by Dr. Gaines Liu, the Tea Experiment Station, Kweichow, China. The types are deposited as indicated under each species.

Bryocoris convexicollis sp. nov. (Fig. 1)

FEMALE. Body elongate, length 4 mm., width 1.5 mm., clothed with pale fine simple pubescence, color principally black, shining.

Head: vertical, small, width across eyes 0.6 mm., length 0.6 mm.; vertex convex, with 0.35 mm., very tenuously marginate, lateral margins near eyes pale, longitudinal median sulcus shallow and broad; frons convex, depressed immediately above base of clypeus; clypeus prominent; juga flat, slightly paler; genae medium; gula very short. Eyes small, almost contiguous with apex of pronotum. Rostrum flavous, short, not reaching apex of mesosternum.

Antennae: inserted at middle of interior margin of eyes, linear, fuscous; segment I, length 0.34 mm., slightly paler; II, length 1.12 mm., slightly thickened at apex, apex nearly as thick as I; III, length 0.5 mm.; IV, length 0.43 mm., the last two segments more slender than II.

Pronotum, length 0.74 mm., width at base 1.25 mm., at apex 0.45 mm., impunctate, strongly convex posteriorly; collar, base before scutellum and posterior angles flavous; posterior and lateral margins straight, collar as thick as antennal segment I, calli hardly distinguished, slightly impressed posteriorly. Mesoscutum covered. Scutellum, length 0.43 mm., width at base 0.6 mm., moderately tumid, lateral margins widely pale. Xyphus very concave, lateral margins elevated. Mesosternum somewhat convex, with middle part depressed; ostiolar peritreme creamy pale, anterior margin strongly elevated. Venter ferruginous-fuscous.

Hemelytra: pale, semitransparent; embolar margin narrowly, apex of corium broadly, and apical half of cubital vein, base of clavus broadly, interior margin and commissure narrowly, apex of cuneus, membrane with apical margin very narrowly and veins, fuscous. Corium, length 1.51 mm., lateral margin slightly curvate. Cuneus, length 0.95 mm., width at base 0.6 mm.

Legs: slender, moderately long, flavous, apical third of femora, tibiæ, apex of tarsi and claws lightly ferruginous.

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Allied to *B. pteridis* Fall. but differs in the larger size, more convex pronotum, and the coloration of antennae, clavus, scutellum and hind femora.

Holotype: ♀ ,Omei San, Szechuan, July, 1932, collected by G. Liu, in the author's collection.

Deraeocoris nigropectus sp. nov.

FEMALE. Body oblong, length 4.16 mm., width 2.04 mm.; above glabrous, ochraceous with dark markings, coarsely nigropunctate except on head and scutellum; beneath black, clothed with fine golden pubescence.

Head: slightly inclining, smooth, shining, lightly fulvous, length 0.65 mm., width 0.87 mm.; vertex, width 0.39 mm., very tenuously marginate, margin narrowly black; clypeus prominent, confluent with frons, fuscous at apex, Rostrum, length 1.52 mm., reaching apex of intermediate coxae, segment I attaining anterior margin of xyphus, segment IV fuscous.

Antennae: black, sparingly clothed with fine silvery hairs; segment I, length 0.43 mm., width 0.17 mm.; II, length 1.06 mm., slightly incrassate at apex, somewhat paler at basal half; segment III and IV missing.

Pronotum, length 1.10 mm., width at base 1.70 mm., at apex 0.68 mm., coarsely punctate; a large black spot on each side of disk confluent with each other anteriorly, thus forming an incomplete longitudinal median line; lateral margins straight, ecarinate, posterior margin somewhat rounded; collar, thickness 0.13 mm., shining. Scutellum, width at base 0.76 mm., length 0.49 mm., impunctate, black, lateral margins widely flavous. Xyphus with disk tending to dark, planate, sides elevated. Sternum dark, ostiolar peritreme pale. Venter uniformly black, clothed with fine golden hairs.

Hemelytra: width 1.04 mm., length 3.16 mm., coarsely black punctate; embolar margins moderately curvate, length 2.04 mm.; cuneus declivent, length 0.65 mm., width at base 0.74 mm., apex fuscous and smooth.

Legs: somewhat cylindrical; femora with two subapical fuscous rings; tibia with base, apex, and a wide ring at middle fuscous, weakly spinose; tarsi fuscous at apex, claws dark.

Allied to *D. orientalis* (Dist.) and *D. signatus* (Dist.) but distinguished from the former by the somewhat unicolorous hemelytra and the uniformly black antennal segment I, and from the latter by the shorter antennal segment I and more coarsely punctate hemelytra.

Holotype: ♀ , Pingloo, Kwangsi, May, 1933; 1 ♀ Paratype, Taipingshien, Anhwei, Oct., 1932; both were collected by G. Liu, in the author's collection.

Deraeocoris anhwenicus sp. nov.

FEMALE. Body oblong, length 4.75 mm., width 1.95 mm., above glabrous, fulvous, coarsely fuscous punctate (except head and scutellum), beneath ferruginous.

Head: slightly inclining, length 0.61 mm., width 0.82 mm.; vertex, width 0.35 mm., immarginate; clypeus moderately prominent, confluent

at base. Eyes reddish. Rostrum, length 1.54 mm., surpassing middle of intermediate coxae, fuscous at apex, segment I barely surpassing base of head.

Antennae: concolorous with body; segment I, length 0.42 mm., thickness 0.07 mm.; II, length 1 mm., clothed sparingly with rather long silvery hairs, slightly incrassate and ferruginous at apex; III, a little more slender than II; IV missing.

Pronotum, length 1.04 mm., width at base 1.65 mm., at apex 0.61 mm., coarsely punctate, posterior margin slightly rounded, lateral margins straight, ecarinate, disk posteriorly moderately convex; calli smooth, lightly fuscous, more or less confluent; collar, thickness 0.07 mm., concolorous, shining. Scutellum, length 0.52 mm., width at base 0.78 mm., dark brown, lateral margins paler. Xyphus with sides elevated. Ostiolar peritreme flavous. Venter clothed with simple flavous hairs.

Hemelytra: fuscous-punctate, longly surpassing apex of abdomen; embolar margins moderately curvate, length 1.08 mm.; cuneus, length 0.65 mm., width at base 0.74 mm., apex smooth; membrane and veins concolorous.

Legs: testaceous, two subapical rings on femora, apex, sub-base and middle of tibia, apex of tarsi and claws, fuscous to blackish; tibia with very few testaceous spinules.

Allied to *D. nigropectus*, n. sp., but distinguished readily by the concolorous pectus, more or less unicolorous pronotum, immarginate vertex and sparingly spinulose tibiae.

Holotype: ♀, Taipingshien, Anhwei, Oct., 1932, collected by G. Liu, in the author's collection.

Deraeocoris alticallus sp. nov

FEMALE. Body oblong, length 5.15 mm., width 2.25 mm., tawny brown, glabrous, coarsely punctate except on head and scutellum.

Head: slightly inclining, length 0.71 mm., width 0.91 mm.; vertex, width 0.42 mm., very tenuously marginate; clypeus moderately prominent concolorous, confluent with frons at base. Eyes not contiguous with pronotum, fuscous, tinged with reddish. Rostrum, length 2.15 mm., barely reaching middle of hind coxae; segment I surpassing base of head, IV fuscous at apex.

Antennae: linear, flavous, clothed with pubescent hairs; segment I, length 0.65 mm., thickness 0.09 mm., cylindrical, tinged with reddish; II, linear or very slightly thickened at apex, length 1.91 mm., distinctly more slender than I, fuscous on apical fourth; III, length 0.82 mm., fuscous at extreme apex; IV, length 0.74 mm., slightly fuscous on apical third; the last two segments as thick as base of II.

Pronotum, length 1.17 mm., width at base 1.91 mm., at apex 0.78 mm., coarsely and rather closely punctate, lateral margins straight, posterior margin somewhat rounded, slightly marginate; calli strongly elevated, confluent with each other at middle, smooth; collar, thickness 0.06 mm., shining. Mesoscutum nearly covered. Scutellum, width at base 0.87 mm.,

length 0.74 mm., smooth, strongly convex. Xyphus with lateral margins elevated. Mesosternum concolorous, with a very tenuous longitudinal median line reddish. Venter slightly reddish, clothed with simple golden pubescence.

Hemelytra: longly surpassing apex of abdomen, coarsely punctate; embolar margins moderately curved, length 2.38 mm.; cuneus, length 0.74 mm., width at base 0.74 mm., declivent, smooth except on interior angle; membrane and veins fuscous, finely but distinctly rugulose.

Legs: cylindrical, ferruginous, base of femora flavous, spinules on tibia concolorous, tarsi flavous, claws fuscous.

Distinguished from *D. martini* (Put.) by the color of head, pronotum, and legs; from *D. anhwenicus,* n. sp. by the longer antennae, elevated calli, raised scutellum and very tenuously marginate vertex; and from *D. flavidus* Popp. by the larger size and wider vertex.

Holotype: ♀, Chungking, Szechuan, July, 1932, collected by G. Liu. in the author's collection.

Deraeocoris montanus sp. nov.

FEMALE. Body oblong, length 6.06 mm., width 2.42 mm., reddish to dark reddish brown, clothed both above and below with simple long flavous hairs; pronotum and hemelytra coarsely punctate.

Head: slightly inclining, length 0.56 mm., width 0.91 mm., concolorous with body, sometimes brownish, with clypeus and disk of frons tending to fuscous; vertex, width 0.43 mm., tenuously marginate; base of clypeus slightly discrete with frons. Rostrum, length 1.86 mm., reaching middle of intermediate coxae, darkened at apex.

Antennae: segment I relatively long, length 0.82 mm., thickness 0.09 mm., cylindrical, reddish to fuscous; II, length 2.08 mm., thickness 0.07 mm., linear, flavous, fuscous at apex; III, length 0.82 mm., thickness 0.05 mm., flavous, apex lightly darkened; IV, length 0.65 mm., fuscous apically.

Pronotum, length 1.08 mm., width at base 1.91 mm., at apex 0.78 mm., coarsely punctate, lateral margins straight, ecarinate, posterior margin slightly rounded; calli conspicuous, smooth, confluent; collar, thickness 0.06 mm., shining. Scutellum, width 0.95 mm., length 0.65 mm., impunctate, moderately convex, lateral margins widely pale. Xyphus with sides elevated, disk concave. Mesosternum flavous to piceous, ostiolar peritreme paler. Venter reddish to piceous.

Hemelytra: coarsely punctate, longly surpassing apex of abdomen; embolar margins moderately curvate, length 2.47 mm.; cuneus reddish to dark red, length 0.91 mm., width at base 0.65 mm., punctures obsolete; membrane fumose, finely rugulose, veins reddish to ferruginous.

Legs: flavous, apical half of femora, tibia (sometimes only at base), apex of tarsi and claws reddish to fuscous, tibiae without spines.

Allied to *D. pilipes* (Reut.) but distinguished by the slender and shining collar; the body is unicolorously reddish brown to fuscous and clothed all over with simple hairs.

Holotype: ♀, Mt. Omei, Szechuan, Sept. 21, 1938, collected by C. S. Tsi, in the author's collection. *Allotype:* ♂, Taichow, July 4, 1935, collector unknown, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* ♂, Taichow, July 8, 1935, collector unknown.

Aretas chinensis sp. nov. (Fig. 2 a, b)

MALE. Body oblong, length 3.03 mm., width 1.4 mm., yellow with red and black markings, clothed with fine concolorous simple hairs.

Head: vertical, width 0.68 mm., length 0.52 mm.; vertex, width at front margins of eyes 0.22 mm., obsoletely marginate; frons fuscous anteriorly, strongly convex; clypeus moderately prominent, fuscous at base, discrete with frons; juga and lora fuscous. Eyes large, reddish, coarsely granulate. Rostrum, length 0.87 mm., slightly surpassing hind margin of mesosternum.

Antennae: inserted at the middle of anterior margins of eyes; segment I, length 0.22 mm., thickness 0.11 mm., black; II, length 1.3 mm., cylindrical, as thick as I, black at extreme base; III, length 0.43 mm.; IV, length 0.4 mm.; both III and IV much more slender than II and fuscous at extreme base. .

Pronotum, length 0.37 mm., width at base 1.05 mm., at apex 0.43 mm., basal margin slightly sinuate, lateral margins straight; a very fine transverse impressed line at anterior fifth forming a rather broad and indistinct collar, and another broader one at posterior two-fifths; a broad longitudinal band on each side reddish and tinged fuscous anteriorly. Mesoscutum broadly exposed, sides reddish. Scutellum, length 0.38 mm., width at base 0.46 mm., a longitudinal median line reddish. Ventral side unicolorous, genital segments fuscous. Male claspers as shown in the figure.

Hemelytra: embolar margins nearly straight; corium length 1.62 mm., base and apex red; clavus with basal half and a subapical spot red; cuneus, length 0.51 mm., width at base 0.33 mm., tinged reddish; membrane semitransparent, finely rugulose, veins reddish.

Legs: concolorous with body, posterior femora thickened, tibia sparsely but longly spinulose, spinules concolorous.

In the key by Dr. Knight (1937) this species runs down to *A. bakeri* Knight, but differs in color markings, size of body, and structure of the male genital claspers.

Holotype: ♀, Shin Kai Si, Mt. Omei, Szechuan, alt. 4,000 ft., date unknown, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C.

Lygus minutus sp. nov.

MALE. Body oval, length 3 mm., width 1.6 mm., yellowish (probably greenish when alive), subtranslucent, clothed with concolorous simple hairs.

Head: subvertical, length 0.70 mm., width 0.74 mm., apex below apical margin of eyes black, somewhat produced; vertex, width 0.32 mm., margination obsolete; clypeus not prominent, confluent with frons; lorum prominent; gula short; eyes large, hind margins forming straight line with base of vertex. Rostrum, length 1.51 mm., distinctly surpassing apex of hind-coxae, apex fuscous.

Antennae: inserted beyond the middle of interior margin of eyes; segment I, length 0.43 mm.; II, length 1.25 mm., black slightly incrassate at apex; segment III and IV missing.

Pronotum, length 0.74 mm., width at base 1.26 mm., at apex behind collar 0.64 mm., obsoletely punctate, basal margin moderately rounded, sides straight, calli small and smooth, collar as thick as base of second antennal segment. Mesoscutum covered. Scutellum, length 0.43 mm., width at base 0.56 mm., uniformly black. Venter ferrugino-fuscous.

Hemelytra: embolar margin slightly curvate, black; corium, length 1.51 mm., subapically fuscous; claval suture depressed, interior margin tinged fuscous; cuneus, length 0.43 mm., width at base 0.55 mm., basal half fuscous; membrane fumate, veins fuscous.

Legs: cylindrical, concolorous with body and devoid of fuscous markings, spinules on tibia concolorous, tarsi with apex fuscous.

As the margination of vertex is very obsolete this species must be referred to the subgenus *Lygocoris*. It is easily distinguished from the known species by its small size, and the black color of the scutellum and the apex of the head.

Holotype: ♂ , Chingchengshan, Szechuan, July, 1932, collected by G. Liu, in the author's collection.

Lygus szechuanensis sp nov.

MALE. Body oval, length 4.50 mm., width 2.82 mm., brown-black to black, shining, clothed with flavous simple hairs.

Head: inclined, width 1.04 mm., length 0.87 mm.; vertex, width 0.24 mm., margin elevated, ferruginous posteriorly; clypeus moderately prominent, confluent with frons; genae low, gula moderately long. Rostrum, length 2.08 mm., barely reaching apex of hind coxae, ferruginous, apex fuscous, segment I not reaching apex of xyphus.

Antennae: inserted near apex of interior margin of eyes; segment I, length 0.56 mm., black, sometimes paler; II, length 1.95 mm., distinctly incrassate at apex, sometimes flavous at base; III, length 0.73 mm., paler at base; IV, length 0.70 mm.; last two segments very slender.

Pronotum, length 0.95 mm., width at base 1.86 mm., at apex behind collar 0.77 mm., remotely and coarsely punctate; calli smooth, confluent anteriorly, base before scutellum truncate, sides straight. Mesoscutum partly covered. Scutellum, length 0.65 mm., width at base 1.08 mm., impunctate, but transversely strigose. Xyphus with lateral margins elevated. Ventral side with ostiolar peritreme and the margin of pleura next to coxae flavous.

Hemelytra; coarsely punctate; embolar margins moderately curvate,

length 2.17 mm.; cuneus, length 0.87 mm., width at base 0.78 mm., declivent; membrane and veins fuscous.

Legs: femora somewhat incrassate, with a subapical ring flavous, sometimes the ring obsolete; tibia with a sub-basal ring and apical half (apex excepted) flavous, spinules fuscous, destitute of black spots at base; tarsal segment II more than twice as long as I, segment III the longest, apex fuscous.

This species must be referred to the subgenus *Lygus*. It may be easily distinguished by the uniformly black body and the incrassate second antennal segment.

Holotype: ♂, Chingchengshan, Szechuan, July, 1932, collected by G. Liu, in the author's collection. Paratype: 4 males, taken with the type; 1 male, Mt. Omei, alt. 4,400 ft., Szechuan (D. C. Graham); 1 male, O-Er. 26 mi. north of Li Fan, Szechuan, alt. 9,000 ft., 1933 (D. C. Graham).

Lygus szechuanensis var. ruficephalus nov.

Structurally not differing from typical *szechuanensis*, but head (except clypeus), antennal segment I and base of II, anterior part of pronotum, xyphus, propleura, episternum, rostrum (except apex), anterior coxae, femora, and tibiae, reddish.

Type: ♂, O-Er, 26 mi. north of Li Fan, Szechuan, alt. 9,000 ft., 1933, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* ♂ and ♀, same data as type; ♂, near Muping, July, 1929 (D. C. Graham); ♂ and ♀, Chingchengshan, Szechuan, July, 1932 (G. Liu); ♂, Pei Bay, Szechuan, July, 1932 (G. Liu); ♂, Kingfoo Shan, Szechuan, Aug., 1932, (G. Liu); and ♂, Mt. Omei, Szechuan, Sept. 15, 1938 (K. F. Chen).

Eurystylus luteus sp. nov.

FEMALE. Body oblong, quite robust, length 6.5 mm., width 2.77 mm., flavous, mottled with fuscous; clothed with pale to golden tomentose pubescence, intermixed with minute simple golden and fuscous hairs.

Head: inclining, length 1.08 mm., width 1.17 mm.; vertex, width 0.52 mm., immarginate; clypeus prominent, strongly compressed, discrete with frons, a spot at base of antennae black. Eyes black, hind margin pale, collum behind eyes shining black. Rostrum, length 1.95 mm., barely reaching apex of intermediate coxae, segment I surpassing anterior margin of pronotum, III and IV nigro-fuscous.

Antennae: segment I, length 1.12 mm., width 0.34 mm., distinctly compressed, constricted at base, clothed with pale tomentose hairs, intermixed with simple fuscous hairs, ferruginous, spotted with pale; II, length 2.25 mm., incrassate at apex, width at apex 0.25 mm., ferruginous, pale at extreme base, gradually darkened at apex, clothed with minute pale pubescence; III, length 0.73 mm., width 0.11 mm.; IV, length 0.57 mm., width 0.07 mm.; the last two segments dark, with base pale, clothed with minute pale pubescence.

Pronotum, length 1.82 mm., width at base 2.38 mm., at apex 1 mm., lateral margins straight, posterior margin rounded, nearly straight or slightly sinuate before base of scutellum, strongly declivent; two subapical transverse spots and two short longitudinal lines on posterior portion fuscous; collar, thickness 0.22 mm., narrowed at sides. Mesoscutum partly covered. Scutellum, length 1.38 mm., width 1.30 mm. Mesosternum chiefly fuscous at middle. Venter flavous, with a sublateral line on each side and vagina exterior dark.

Hemelytra: longly surpassing apex of abdomen; embolar margin nearly straight, length 3.16 mm.; interior apical half of corium, base and apex of cuneus chiefly fuscous; cuneus, length 1.04 mm., width at base 1.04 mm., strongly declivent; membrane transparent, rugulose apically, apical margin fumose, veins and an oblique line behind areole fuscous.

Legs: cylindrical, comparatively short, ferruginous, spotted with flavous, tending to uniformly ferruginous to fuscous apically; spinules on tibia ferruginous to fuscous.

Allied to *E. sauteri* Popp. but differs in the larger size and different coloration. Posterior margin of pronotum is not broadly sinuate and more than twice as wide as apex. Second antennal segment is about twice as long as the first and scarcely three times as long as the third.

Holotype: ♀, Taipingshien, Anhwei, Oct., 1932, collected by G. Liu, in the author's collection. *Paratype:* 2 ♀ ♀, taken with the type.

Adelphocoris glaucus sp. nov.

FEMALE. Body elongate, length 8.14 mm., width 2.64 mm., bluish green, shining, smooth, sparsely clothed with fine sericeous pubescence, intermixed with simple fuscous hairs above and fine pale hairs beneath; color beneath uniformly paler than above.

Head: inclining, width 1.08 mm., length 0.9 mm.; vertex, width 0.44 mm., slenderly marginate, with a shallow median longitudinal sulcus; frons obsoletely obliquely strigose; clypeus prominent, bending downward, confluent with frons, facial angle forming a right angle. Rostrum, length 2.80 mm., surpassing apex of hind-coxae, apical two-thirds flavous, apex fuscous, segment I reaching middle of xyphus.

Antennae: linear, inserted at apical third of interior margin of eyes; segment I, length 0.87 mm., flavous at extreme apex; segment II, length 2.94 mm., flavous at apical half, tending to fuscous or black on apical fourth; segment III, length 1.38 mm., progressively fuscous; segment IV missing.

Pronotum, length 1.29 mm., width at base 2.16 mm., at apex behind collar 0.87 mm., disk posteriorly finely transversely rugulose, basal margin moderately rounded, lateral margins straight; collar somewhat opaque, as thick as antennal segment II; calli small and distinct. Mesoscutum exposed. Scutellum, length 1.08 mm., width at base 1.08 mm., finely transversely rugulose. Xyphus concave, lateral margins elevated.

Hemelytra: embolar margins nearly parallel, length 3.89 mm.;

cuneus, length 1.40 mm., width at base 0.87 mm., cuneal fracture distinct; membrane light fuscous, veins flavous to greenish.

Legs: of moderate length, color of coxae slightly pallid; femora somewhat cylindrical, extreme apex sometimes flavous; tibia progressively flavous at apex, spinules ferruginous; tarsi flavous, segment III longest, fuscous at apex, claws ferruginous.

Male more slender than female but similar in color and pubescence.

This species can easily be distinguished from the known species by its solid, uniform bluish green color; size and color suggestive of *Allorhinocoris flavous* Salhb., but the structure of the head is of the Adelphocoris type.

Holotype: ♀, Mt. Omei, Szechuan, alt. 11,000 ft., Aug. 19, 1934, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Allotype:* ♂, taken with the type. *Paratype:* male and female, taken with the type; male, Wei Chow (65 mi. N. W. Chehgtu), Szechuan, alt. 5,500-9,000 ft., Aug. 5, 1910; and male, Yachow, Szechuan, alt. 2,200-4,000 ft.; Aug. 25, 1930 (D. C. Graham).

Phytocoris knighti sp. nov. (Fig. 3)

MALE. Body elongate, length 7.66 mm., width 2.34 mm., principally fuscous, above conspurcate and varied with green areas; sparsely clothed with sericeous pubescence and intermixed with simple hairs, the latter concolorous with their location.

Head: subvertical, width 1.08 mm., length 0.91 mm.; vertex, width 0.30 mm., immarginate, green; frons fuscous, clypeus moderately prominent, blackish, confluent with frons; lorum tumid, genae very low, gula short; eyes large and prominent, coarsely granulate. Rostrum, length 2.86 mm., surpassing apex of hind coxae, ferruginous, darker at apex, paler at the joints; apex of segment I green, barely reaching apex of xyphus.

Antennae: linear, inserted just below the middle of interior margin of eyes; segment I, length 1.3 mm., thickest near base, fuscous, mottled with greenish; II, length 2.94 mm., blackish, base and middle widely annulated with greenish white; III and IV missing.

Pronotum, length 1.08 mm., width at base 1.95 mm., at apex behind collar 0.82 mm., black, basal margin narrowly and middle of disk through collar very broadly greenish; base moderately rounded, sides slightly sinuate behind middle; collar as thick as antennal segment I; calli small. Mesoscutum exposed, with sides green and middle fuscous. Scutellum, length 0.95 mm., width at base 1.08 mm., green, a small spot at apical third of lateral margins fuscous. Xyphus green, base fuscous, lateral margins elevated, disk planate. Ventral side uniformly fuscous.

Hemelytra: fuscous to black, a series of small spots along embolar margin, a large spot beyond the middle of corium, apex of corium, base, apex, and interior margin of clavus, and basal half of cuneus, greenish; corium, length 3.90 mm., embolar margin straight; cuneus, length 1.3

mm., width at base 0.87 mm., membrane fumate, spotted with fuscous, veins greenish and fuscous.

Legs: long, femora fuscous, spotted greenish, pale at middle; tibia fuscous, basal half of hind pair with two broad bands greenish white, spinules ferruginous.

Distinguished from known species by the large and prominent eyes, the narrow vertex, and conspicuous coloration of the body.

Holotype: ♂, Mt. Omei, alt. 11,000 ft., Szechuan, Aug., 1934, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* males, taken with the type. Named in honor of Dr. H. H. Knight who has been assisting the writer in his studies on Miridae.

PLATE I

Fig. 1, *Bryocoris convexicollis* sp. nov.

Fig. 2, *Aretas chinensis* sp. nov., front view of head; a, left genital clasper; b, right genital clasper.

Fig. 3, *Phytocoris knighti* sp. nov.

PLATE I

1

2

2a   2b   3
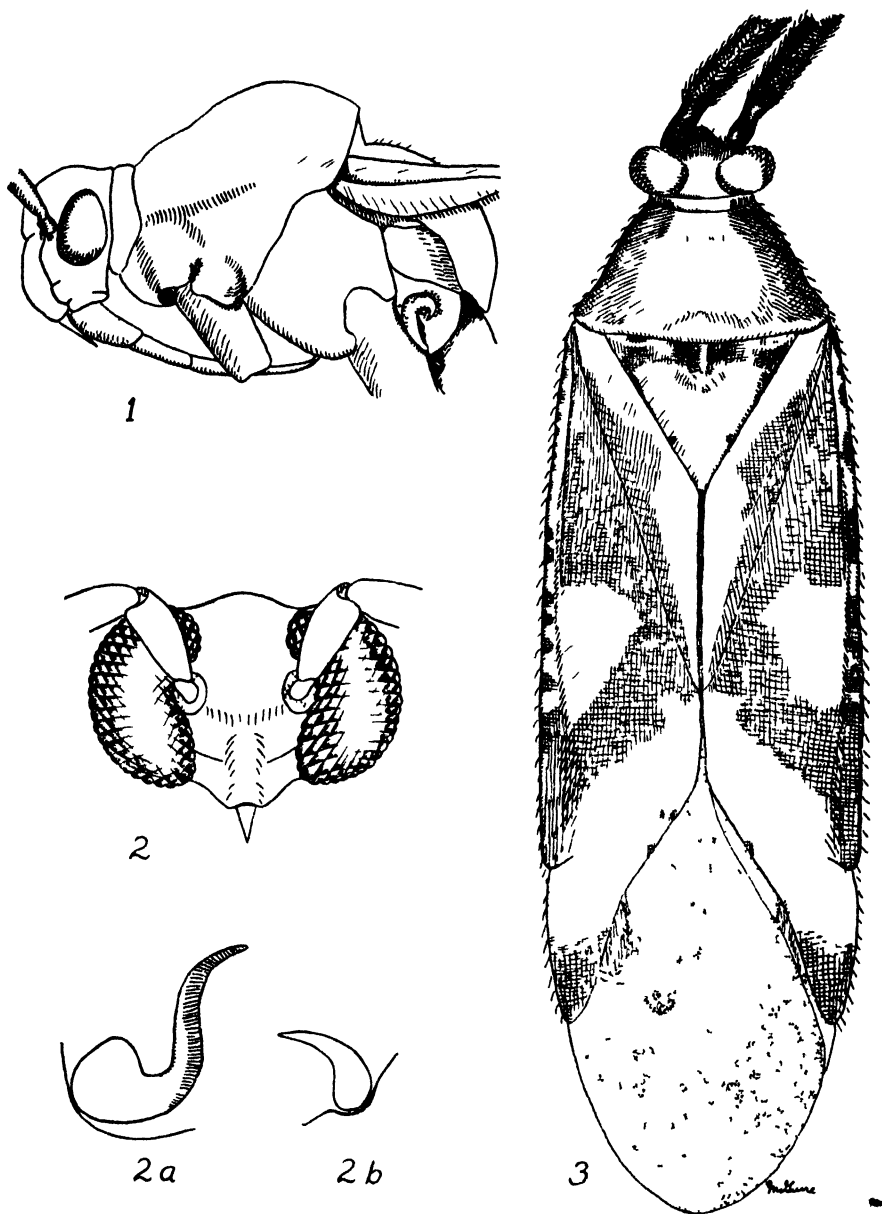
THE DETERMINATION OF URONIC GROUPS IN SOILS AND PLANT MATERIALS

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During the past decade it has become apparent that polyuronides and uronic groupings are widely distributed in plant materials, decomposed residues, and soils. In general the uronic content is low, and although determination is a simple matter, the problems to be faced are not the same as when carried out on pure polyuronides, or compounds such as pectin and gums with a high uronic content. The principle of the determination was first elucidated by Lefèvre and Tollens (3), and depends on the measurement of the carbon dioxide evolved on prolonged boiling with 12 per cent hydrochloric acid. Carboxyl groups, other than uronic, occurring in plant materials, do not appear to be decarboxylated under these conditions. The yield of carbon dioxide from pure uronic acids or polyuronides is virtually quantitative. Small quantities of carbon dioxide are evolved, however, from certain non-uronic groups, notably from hexosans. The amount is small, but in materials of high polysaccharide content and low uronic content, the error from these sources is not inappreciable. It is desirable, therefore, that in such cases the determination should be carried out under optimum conditions for the decarboxylation of uronic groups and with a fully standardized procedure that holds constant for any material the error from non-uronic sources.

The apparatus to be described involves no new principles, but the modifications introduced are such that the minimum of attention is required. Inasmuch as the period of determination is ordinarily 4½-5 hours there are obvious advantages in being able to leave the apparatus with complete safety.

DESCRIPTION OF APPARATUS

The essential parts of the apparatus are similar to those employed by other workers (2) (4). The modifications chiefly concern the methods of control of aeration rate and bath temperature.

The reaction vessel is a 300-ml. pyrex round bottom flask with a standard ground glass joint (24/40) into which is fitted a 30-cm. reflux condenser of the Allihn type (fig. 1). The air intake is led down the center of the condenser and does not dip below the acid in the flask. The air stream is carried to the Truog tower absorbing system by 7-mm. tubing passing enroute through a small trap containing silver sulfate. The tower, about 60 cm. high, is composed of 17 mm. tubing, into which at one end is sealed a small gooch disc, and is filled about two-thirds full of 5-6-mm.

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glass beads. To facilitate and to obtain uniformity in washing, a constant delivery pipette (200 ml.) is fixed above the bead towers, filled by gravity from a source of CO_2-free water.

Carbon dioxide-free air is drawn through the system by means of a water pump and the rate of flow controlled by capillary tubes. Three capillary controls are provided, one permitting the passage of 1.5-2 l. per hour, another 4-5 l. per hour, and a third, 6-8 l. per hour, with the water pump at full capacity. These controlling capillaries are made by trial from ordinary heavy-walled capillary tubing. One end of each of
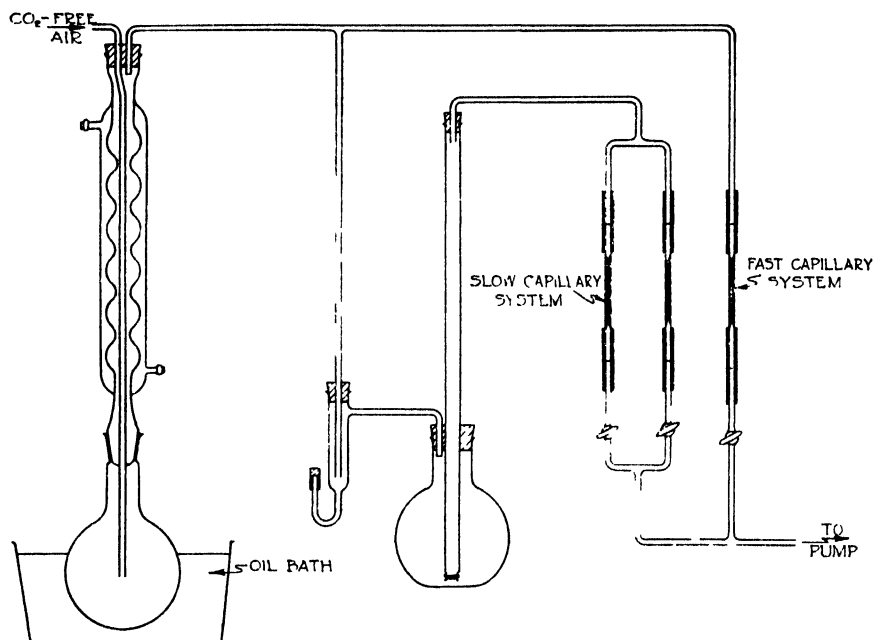
Fig. 1. Diagram of apparatus for the determination of uronic groups in soils and plant materials.

the two slower capillaries is connected through a stop-cock and T-joint to the suction pump guard flask, and the other to the tube leading to the top of the bead towers. The third and fastest capillary is connected to the pump and the aeration system between the condenser and the silver sulfate trap, thus shunting the air stream across the absorbing tower.

The temperature of the reaction flask is maintained by means of an electrically heated oil bath automatically controlled (fig. 2). The thermostat consists of a 100-ml. glass bulb filled with hydrogen sealed to a manometer tube mercury-filled. Two electrodes, one about 12 cm. below the other, are sealed into the arm of the manometer tube. One electrode is to prevent the temperature from exceeding 60° during preliminary aeration, and the other is to maintain the reaction temperature at 135°.

Switches are provided so that one or the other control may be used. When first installed the hydrogen-filled bulb is heated to the desired temperature, and the mercury reservoir attached to the manometer is adjusted for height so that the mercury column makes contact with the lower electrode. The bulb is sealed off so that at 135° the pressure inside equals that of the atmosphere.[3] The heating unit in the bath is connected directly through the thermostat without a relay.

The heating unit is composed of 24 inches of No. 30 nichrome wire wound around two porcelain poles held in a frame of pyrex glass submerged in the oil bath. This controlled heating system has proved very satisfactory in operation.

OPERATION

The sample together with 100 ml. 12 per cent HCl are introduced into the boiling flask and aeration commenced through the rapid capillary system, the lower temperature bath heater being switched on at the same time. After 10 minutes the medium or 3-4 l. pr hour capillary control is used, the air stream then being taken

Fig. 2. Diagram of oil bath temperature control system.

through the bead tower. Fifteen minutes later, 25 ml. 0.1 N. NaOH is introduced quickly into the absorbing flask, the slowest capillary system opened, and the high temperature bath control switch closed. The apparatus then requires no attention until the experiment has run its course. Thirty minutes more are required to reach 135°, and aeration and boiling are continued for 4¾ hours at that temperature.

At the conclusion of the run the heating current is shut off, the absorbing flask is lowered and the top of the bead tower opened so that the alkali drains out. The tower is washed down with several aliquots of CO_2-free water from the automatic delivery pipette, the same total volume being used in each case. Ten ml. neutral N barium chloride is added and the solution back titrated with standard acid using phenolphthalein. Blank determinations are carried out using 100 ml. 12 per cent HC1 only, under precisely the same conditions. The full "operation blank" should not differ from the direct "titration blank" by more than 0.3-0.4 ml. 0.1 N. acid.

DETERMINATION OF URONIC CONTENT OF SOIL ORGANIC MATTER

The uronic content of soils is quite variable and not necessarily proportionate to the total organic matter present. Ten grams is a suitable amount of soil in most cases, though when the organic content is low it may be necessary to take a larger amount. 100 ml. 12 per cent acid is

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[3] Wide fluctuations in atmospheric pressure affect the temperature control and have to be compensated for by slightly raising or lowering the mercury column.

added, and aeration commenced as described above. If the presence of carbonates is suspected, the acid is added several hours before the determination is to be made, and rapid aeration of the suspension begun. The time may be shortened somewhat by heating the flask briefly to a temperature of about 50°, which is insufficient to cause liberation of carbon dioxide from uronic groupings. The carbonate may actually be determined in this operation if desired. No difficulty has been experienced with bumping. The results of duplicate determinations on some soils covering the range ordinarily found are given in Table I.

DETERMINATION OF URONIC CONTENT OF PLANT MATERIALS

With the exception of certain special cases the uronic content of plant materials is low, and small in proportion to other carbohydrate groupings, such as hexosans, which may yield a little CO_2 under the same con-

TABLE I

URONIC CONTENT OF VARIOUS SOILS EXPRESSED AS CARBON DIOXIDE YIELD

Soil type	No.*	Depth	g. CO_2 yield per 100 g. air dry soil	
Edina silt loam	P2-1	0-4"	0.082 ;	0.084
Edina silt loam	P2-6	16-19"	0.016 ;	0.016
Weller silt loam	P4-1	0-1½"	0.114 ;	0.118
Weller silt loam	P4-6	10-13"	0.028 ;	0.030
Ames v. f. sandy loam	206	0-2½"	0.232 ;	0.238
Webster silty clay loam	208	0-6"	0.162 ;	0.162
Leon fine sand	P213-1	0-1½"	0.108 ;	0.110
Leon fine sand	P213-2	1½-3"	0.019 ;	0.021

* Numbers refer to soil sample file, Soils Subsection, Iowa Agricultural Experiment Station.

ditions. Perhaps the most difficult situation arises when it is desired to determine with precision the uronic groups present in cellulose preparations, inasmuch as the uronic-derived CO_2 may be little if any in excess of that derived from the hexosan. Standardization of experimental conditions and operations are essential to hold constant the yield from the latter source. Whereas uronic groups are quantitatively decarboxylated by 12 per cent HCl in 4½-5 hours, CO_2 evolution from hexosans continues apparently indefinitely at a low level. Small variations in the concentration of HCl employed have no effect on the yield of CO_2 from uronic units or the time in which quantitative recovery is obtained, but affect considerably the amount of CO_2 evolved from hexosan groups, as shown by Whistler et al. (6).

The opinion has been expressed that in the case of predominantly hexosan materials, yields of CO_2 of the order of 0.2-0.3 per cent in 5 hours cannot be considered to indicate the presence of any uronic groups (5). Campbell, Hirst, and Young (1) in considering the case of starches put the figure higher, and attached no significance to yields of 0.3-0.5 per cent. The yield from a pure monosaccharide such as glucose is not

necessarily identical with that from a polysaccharide. Glucose has been stated to give 0.40 per cent (1), 0.19 per cent (5), and 0.26 per cent (6). Lower figures have been obtained from cellulose preparations believed to be virtually free of uronic groups. Purified cotton cellulose and ramie cellulose have given figures of 0.1 per cent, purified flax cellulose 0.16 per cent (6), hemp cellulose 0.2 per cent (5), and in this work, purified cotton cellulose 0.10 per cent, esparto cellulose 0.16 per cent, acid-hydrolyzed wheat straw cellulose 0.08 per cent, and acid-hydrolyzed chinese hemp cellulose 0.07 per cent. There seems to be clear evidence that the yield of CO_2 from glucose is greater than that from cellulose, even though the conditions employed by the various investigators have not been identical. The disturbing effect of the hexosan groups in pure cellulose may therefore have been somewhat over-estimated.

Other hexosans have been little investigated. The yield from monosaccharides other than glucose appears to be considerably higher than from that sugar (1). Fructose undoubtedly yields appreciable amounts of CO_2 (*circa* 0.6 per cent) and sucrose also by reason of its fructose component.

Little is known of the behavior of the pentoses and pentose polysaccharides. Both xylose and arabinose have been reported to yield CO_2 in excess of that given by glucose (1). The relative reduction in CO_2 evolution from xylan-containing and xylan-free celluloses as a result of dilute acid hydrolysis suggests that the xylan fraction of plant celluloses may have a more serious disturbing effect in uronic determinations than the hexosan component.

Uronic determinations on cellulose preparations should desirably be made on samples that have not been dried by heat. Chemically treated samples should invariably be treated wet and a moisture determination carried out separately. Prior knowledge of the moisture content is essential in order that the acid concentration may be adjusted to 12 per cent. The equivalent of 2.5-5 g. dry cellulose should ordinarily be employed, and larger amounts may be desirable if the uronic content is low.

The yields of CO_2 from various isolated and treated celluloses are given in Table II. It will be seen that the CO_2 yield of many natural celluloses isolated by a standard procedure is quite variable and often considerably above that likely to be obtained from non-uronic sources.

Further evidence of the presence of uronic groups in certain isolated celluloses was obtained in a study of the rate of CO_2 evolution during the period of boiling with acid. The absorbing system was changed every 10 minutes during the first hour, every 15 minutes in the second hour and thereafter at thirty-minute intervals. Uronic groups occurring in a polyuronide, such as pectin, are decarboxylated in a characteristic manner, the curve for rate of evolution exhibiting a sharp peak between 20 and 30 minutes from time of boiling. Slightly less than 50 per cent of the final CO_2 yield was obtained in the first hour (fig. 3). A similar sharp peak was found in the rate of evolution from fructose and sucrose, the maximum, however, being obtained earlier. Glucose behaves in a
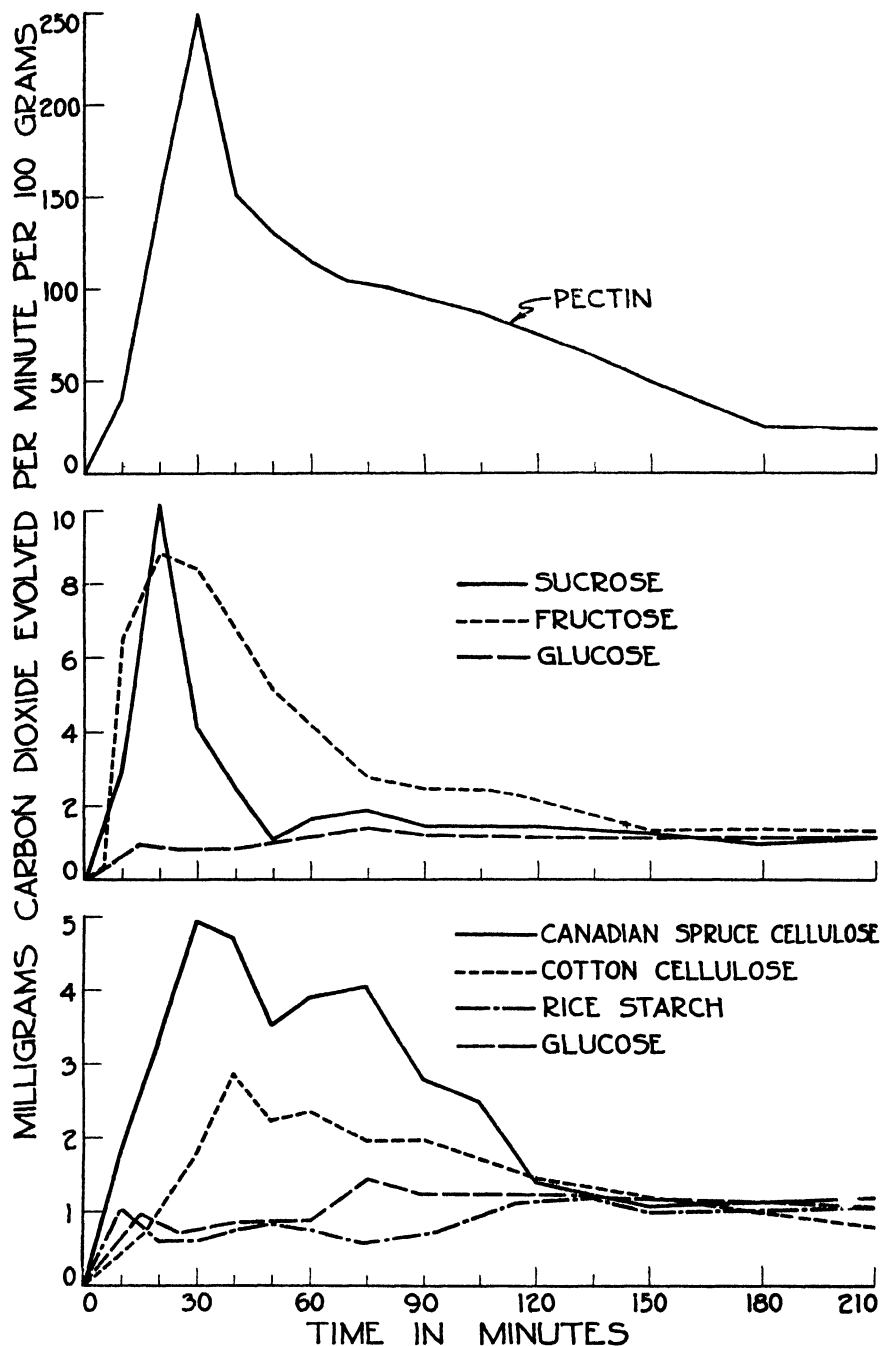
Fig. 3. Rate of evolution of CO_2 from various substances.

TABLE II

CARBON DIOXIDE YIELD FROM VARIOUS CELLULOSE PREPARATIONS

Expressed as g. CO_2 per 100 g. Oven Dry Cellulose

Cellulose	CO_2	Cellulose	CO_2
Cotton (raw)	0.41	English oak‡	0.54
Cotton (purified)*	0.10	Canadian spruce‡	0.42
" (fermented)	0.21	Italian hemp‡	0.19
Cotton oxycellulose (hypo-chlorite)	0.52	Silver fir‡	0.45
" oxycellulose (hypo-bromite)	0.86	Rye grass (mature)‡	0.32
" oxycellulose (dichro-mate)	1.52	Jute‡	0.78
Wheat straw‡	0.30	Sisal‡	0.82
" " (oven dried)	0.44	Esparto	0.16
" " (acid hydro-lyzed)†	0.08	Ramie‡	0.36
Chinese hemp‡	0.37	Flax‡	0.30
" " (acid hydro-lyzed)†	0.07	Alkali pulp (unbleached)	0.41

* Boiled successively with 1 per cent NaOH and 1 per cent HCl.
† Boiled with 5 per cent sulfuric acid for 1 hour.
‡ Isolated by alternate chlorination in suspension and extraction with hot 3 per cent neutral sodium sulfite until lignin-free.

different manner, evolution being at a slightly higher rate in the second hour than the first. An ill-defined peak seemed to occur at about 75 minutes, and thereafter CO_2 was evolved at a steady rate for many hours. The uronic peak occurring in the neighborhood of 20 minutes was detectable in the rate of CO_2 evolution from structural celluloses such as that from Canadian spruce (fig. 3) and to a lesser extent also in cotton cellulose. The former exhibited also a smaller maximum at about 75 minutes probably due to hexosan material. No uronic peak was detectable from rice starch.

The figures obtained in rate of CO_2 evolution experiments such as these have no absolute value since the CO_2 absorbed in short time intervals is in part a characteristic of the size of apparatus in relation to the sample, the rate of aeration, etc. To obtain significant amounts of CO_2 in brief intervals the sample size has to be substantially increased over that of normal determinations. In these experiments the amounts employed were the equivalent of 10 g. dry plant cellulose, 18 g. cotton, and 15-20 g. of the sugars and starch, as contrasted with only 1 g. pectin. The general procedure, however, may be used to establish the probable presence or absence of uronic groups in substances giving low yields of CO_2, and can be employed to supplement the differential method suggested by Whistler et al. (6) which is only applicable if the uronic groups are acid-hydrolyzable and the disturbing hexosan or other groups, acid-resistant.

SUMMARY

1. The construction and operation of an apparatus for the determination of uronic groups in materials of low uronic content, such as soils

and plant materials, is described. Automatic control of the reaction temperature and aeration rate is provided so that the apparatus once started requires no attention.

2. The determination of uronic groups in cellulose preparations is subject to error caused by CO_2 evolution from hexosan groups. The yield from this source, however, is probably lower than that from glucose. Evidence of the presence of true uronic groups in cellulose preparations or similar materials may be obtained by ascertaining the rate of liberation of CO_2. A characteristic distribution curve with a sharp peak within the first 30 minutes is given by uronic-containing compounds.

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THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

I. GLUCOSE CARBON PARTITION[1]

G. Semeniuk[2]

INTRODUCTION

Chaetomium funicola Cke., like other Chaetomium species, was frequently found associated with cellulose materials. This fact suggested an investigation of the possible use of *C. funicola* in commercial utilization of cellulose waste products. Studies were initiated to ascertain (1) the nature and amounts of products produced and accumulated in glucose dissimilation and (2) the mechanism of glucose dissimilation, particularly the nature of the intermediate products formed. The results of a preliminary study pertaining to the first of these points are set forth in the present report and will be followed by further studies.

Literature on the catabolic activity of *C. funicola* was confined to the single work of Lowell (3) who found small amounts of acetic acid to be the only product of the volatile acid fraction when this fungus was cultivated on filter paper suspended in a nutrient salt solution. The formation of other chemical fractions was not reported. Birkinshaw *et al.* (2), using an unidentified *Chaetomium* sp., found that small but definite amounts of nonvolatile acids and nonvolatile neutral compounds and only negligible amounts of volatile neutral compounds were produced from glucose in Czapek-Dox solution. Volatile acids, however, were not formed.

With some modifications in the method, the present study of *C. funicola* was carried out in a manner similar to that followed by Birkinshaw and Raistrick (5) for many different fungi. These modifications comprised (1) determination of a proximate serial carbon balance sheet by the analysis of six cultures of different periods of development, and (2) maintenance of a continuous slow aeration of the cultures in contrast to intermittent aeration.

MATERIALS AND METHODS

A culture of *Chaetomium funicola* Cke. isolated from baled cornstalks (6) was used. Subcultures of this fungus were prepared on 275 cc. of

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Czapek-Dox liquid medium contained in each of six 2-liter Erlenmeyer flasks. The composition of this medium was as follows: 1 liter distilled water, 50.0 gms. Pfanstiehl's technical glucose, 2.0 gms. $NaNO_3$, 1.0 gm. KH_2PO_4, 0.5 gm. KCl, 0.5 gm. $MgSO_4 . 7H_2O$, and 0.01 gm. $FeSO_4 . 7H_2O$. Separate sterilization at 15 lbs. steam pressure for 15 minutes was given to the glucose and mixed salt solutions. The medium in each flask was aseptically seeded with 1 cc. of an aqueous ascospore suspension of the fungus. This suspension was obtained from two-week-old cultures of the fungus growing on the usual potato dextrose agar medium contained in 50 cc. Erlenmeyer flasks.

To provide for the constant aeration of each culture and for the determination of the respiratory carbon dioxide and volatile compounds carried away by the air stream, each 2-liter flask containing the seeded medium was attached to an apparatus assembled for this purpose. A single series of glass containers charged with a 40 per cent solution of KOH, soda lime and a 0.001 per cent solution of $HgCL_2$ rendered the incoming air free of carbon dioxide and micro-organisms. The air was then conducted through a series of connected T-tubes to each of the six culture flasks which were provided with steam sterilized units of glass tubing fixed in two-holed rubber stoppers to serve as inlet and outlet air leads. The air inlet tube extended below the surface of the medium while the outlet tube opened just below the rubber stopper. Nonabsorbent cotton was inserted in the exposed ends of these air leads as further precautionary measure against contaminating organisms. The air stream leaving each culture flask was conducted through a system of units for each flask in which carbon dioxide was determined gravimetrically by absorption in 40 per cent solutions of KOH contained in Bowen potash bulbs, and volatile organic compounds were caught in concentrated H_2SO_4. Air was obtained from a centralized compressed air supply and was passed through each culture at a uniformly slow rate of one bubble per second. Phenolphthalein served as an indicator for the renewal of the KOH solutions. The entire apparatus was built about a constant temperature chamber maintained at 28°C. The cultures were placed inside this chamber, whereas the carbon absorption units were placed outside.

For the analysis of a culture of a chosen interval of development, the liquid filtrate was separated from the mycelial mat by decantation at the suction pump through a previously washed, dried, and weighed piece of percale cloth. The mycelial mat was washed several times with hot distilled water and the filtrate was made to 500 cc. volume. The weight of the mycelial mat was obtained by drying at 70°C. for several days followed by 100°C. for one day and maintained in vacuo over concentrated H_2SO_4 until weighed. Fractionation of the liquid filtrate into the various carbon fractions was done as follows: A 400-cc. aliquot part of the filtrate was made acid to congo red with dilute H_2SO_4 and distilled through a long condenser into an ice-cooled container to obtain 250 cc. of distillate. During this distillation the residual liquid was maintained above a volume of approximately, 150 cc. The distillate was then made alkaline to

phenolphthalein with NaOH and further distilled to produce 100 cc. of distillate containing neutral volatile materials. The residues of these two distillations were then combined and tested for acidity to congo red and then subjected to steam distillation to yield the volatile acid fraction in the distillate and the nonvolatile materials in the residue. Two liters of steam distillate were thus obtained. Further subdivision of the nonvolatile residue was made by continuous ether extraction for 48 hours only after the residue had been reduced from a volume of 300 to 400 cc. to approximately 100 cc. with the aid of a vacuum distillation apparatus. With this latter step the excessive frothing was minimized by allowing the nonvolatile residue to enter drop-wise through a long capillary tube into a 1 or 2-liter evacuated distillation flask immersed in a water bath held below 45°C.

Carbon determinations on aliquot parts of the various fractions were made by the method of Osburn and Werkman (9). Carbon dioxide was caught in ascarite and weighed. Care was taken to use only fresh $K_2S_2O_8$ and to maintain good rubber connections in the apparatus. Glucose was determined by the Bertrand modification of the Munson-Walker method. Hydrogen-ion concentration readings were made by the Coleman glass electrode apparatus. Melting points of crystals were obtained with a hot plate fixed on a microscope stage. Correction for thermometer stem exposure (5 inches long) was not applied to the melting point readings.

EXPERIMENTAL RESULTS
THE PARTITION OF GLUCOSE CARBON

The carbon balance sheet for the six *C. funicola* cultures is presented in table I. The data show the dissimilation of nearly 55 per cent of the glucose within the test period of 33 days development. The rate of glucose dissimilation was not constant throughout this period but was greater during the later stages. The main product formed in addition to mycelium was carbon dioxide. Assuming 50 per cent carbon in the mycelium, these two products retained 40.0, 73.4, 85.7, 76.9, 68.6, and 79.5 per cent, respectively, of the carbon in the dissimilated glucose for each of the six cultures analyzed at the successive dates. The remaining carbon in part was accounted for by the difference between the total carbon in the medium and the carbon in the residual glucose. The values obtained were 12.9, 36.2, 9.5, 12.1, 23.3, and 15.0 per cent. The difference in carbon in the sum of these corresponding values from 100 per cent remains unaccounted. The figures for initial total carbon were corrected on the basis of 14-cc. loss in volume on sterilization. The assumption that the oven-dried mycelium contained 50 per cent carbon was based on the findings of Birkinshaw et al. (1, 2, 3, 4) that in over 200 species of fungi examined, 58 per cent showed a mycelial carbon content of 49-53 per cent and that the species of Chaetomium they examined contained 53 per cent carbon in its dried mycelium (2).

The nature of the nonglucose carbon in the residual medium falls mainly with the nonvolatile, nonacidic, and nonreducing fraction. This

TABLE I

CARBON BALANCE SHEET OF GLUCOSE CARBON PARTITION BY *Chaetomium funicola* CKE.

	DAYS OF DEVELOPMENT					
	3	7	11	18	26	33
Initial total carbon in the medium, gms.	5.64	5.70	5.57	5.48	5.50	5.63
Final total carbon in the medium, gms.	5.37	5.26	4.62	4.32	3.50	3.04
Carbon in the medium as glucose, gms. .	5.33	5.01	4.52	4.16	2.88	2.58
Carbon in the medium as products other than glucose, gms.	0.04	0.25	0.19	0.16	0.61	0.46
Carbon in the medium as neutral volatile compounds, gms.	0.003	0.002	0.003	0.002	0.001	0.006
Carbon in the medium as volatile acids, gms.	0.013	0.030	0.024	0.013	0.007	0.003
Carbon evolved as respiratory carbon dioxide, gms.	0.022	0.159	0.378	0.421	0.942	1.389
pH of medium	6.2	6.4	6.8	7.1	7.7	7.1
Weight of mycelium, gms.	0.204	0.693	1.045	1.189	1.711	2.102

fraction occurred throughout the entire tested period of the experiment and was greatest in amount of formation between the eighteenth and the twenty-sixth day of fungous development when it constituted approximately 35 per cent of the glucose dissimilated in that interval. Relatively abundant early formation of this fraction occurred with the 7-day-old culture. Nonsignificant amounts of nonvolatile acids were formed throughout the tested period. The lack of formation of appreciable amounts of such acids was indicated by (a) the progressive change of pH to the alkaline side, (b) the formation of only small amounts of lead precipitates during the clarification process for sugar analysis, and (c) the formation of only very small amounts of amorphous precipitates as calcium salts insoluble in 80 per cent ethyl alcohol (5). Volatile neutral compound formation was negligible. The volatile acid fraction yielded significant small amounts of carbonaceous material. Volatile acids, however, constituted a very small part of this fraction since at no time was more than 5.0 cc. of 0.02 N NaOH required to titrate this fraction to phenolphthalein. The greater part of the carbon in this fraction was attributed to substances readily carried over by steam distillation. These distillates exhibited very faint cloudiness and a slight paraffin-like scum on the surface.

The percentage recovery of total carbon for the different cultures could not be calculated from the data in Table I because no determinations were made for mycelium carbon. By assuming 50 per cent carbon in the mycelium, however, the percentage recovered total carbon as derived from the carbon in the mycelium, the residual medium, and the evolved carbon dioxide would become 98.4, 101.2, 99.2, 97.3, 96.2, and 97.4 per cent, respectively, for the cultures analyzed at the successive dates. The concentrated H_2SO_4 solution in the absorption train of each culture was not

analyzed for the carbon retained because the clear condition of this solution throughout the experimental period was taken as an indication of the absence of any significant amounts of such carbon.

THE FORMATION OF ORGANIC COMPOUNDS WITHIN THE MEDIUM

Variations in substrate coloration as induced by Chaetomium spp. has been noted by Dickson (7) and Tschudy (10). The nature of the coloring material has not been determined. In the present study C. *funicola* rendered Czapek-Dox solution light yellow during the early stages of its development and brownish-orange in the later stages. Ether extraction of the nonvolatile concentrated residue (made acid or alkaline) yielded a yellowish or a brownish-orange ether solution depending upon the age of the culture and to some extent upon the length of the continuous extraction. The color of the residue was not removed appreciably by this extraction. The ether-soluble fraction contained a reddish-brown fatty liquid and solid materials such as yellow crystalline flakes and short crystalline rods (m.p. 113.0° to 113.5°C.). The fatty liquid was found to have a refractive index of 1.5107 at 24.5°C. and a specific gravity of 0.880 at 21.5°C. Distillation at ordinary pressures and in air produced fumes and an odor of burned fat. A yellow liquid distillate was obtained with a refractive index of 1.5017 at 20°C.

Other substances in the medium were detected in the liquid substrates of nonartifically aerated, 18-day-old C. *funicola* cultures developing on 250 cc. Czapek-Dox medium containing 10 per cent sucrose. The liquid substrates from five such cultures contained in 2-liter Erlenmeyer flasks were bulked, acidified to congo red, and concentrated in vacuo. A solidified mass was obtained, which on extraction with ether yielded approximately 0.5 gm. of a reddish-brown material. Fractionation of this material with solvents yielded the substances as shown in Figure 1. Because of the very small amounts of materials on hand, further purification of the various fractions was not followed. The results, however, indicate the formation of different organic substances in the medium.

EFFECT OF AERATION AND AGITATION OF THE MEDIUM

Since in the foregoing experiment the culture medium was only slightly agitated by the slow incoming air and the fungus readily developed into a surface mat, an attempt was made to induce the fungus to grow throughout the medium. For this purpose a 2-liter Erlenmeyer flask was used in which were placed 1,500 cc. of Czapek-Dox solution containing 4 per cent glucose. To it were added aseptically 150 cc. of a liquid culture of similar composition contained in a 2-liter flask in which the organism had developed for a period of three days. A vigorous stream of carbon-dioxide-free air was passed through the medium by means of a four-way-outlet glass tube set deep in the medium. The agitation induced by the air stream was sufficient to keep the medium in constantly slow rotatory motion. In the presence of this agitation the fungus developed profusely throughout the medium as small individual colonies that gradually

REDDISH-BROWN RESIDUE

obtained by ether extraction

Acetone

INSOLUBLE (1)

White crystalline material of small, short. rectangular plates frosted at 120° C. and melted at 215-218° C., leaving a residue. Several crystals melted at 137° C.

SOLUBLE
(Reddish solution)
Acetone evaporated off

Chloroform

SOLUBLE (2)
(Brown solution)
Evaporation of sol·vent yielded brown liquid

INSOLUBLE

Ether

SOLUBLE (3)
(Yellow solution)
With partial removal of ether obtained

INSOLUBLE

Absolute
Ethyl alcohol

INSOLUBLE (4)

Crystalline material with a faint yellow tinge. Part of this material melted at 174° C. and another part at 191° C. Some material melted at 165° C. and other crystalline, colorless material at 180-188° C.

SOLUBLE (8)
(Brown solution)
Evaporation of solvent yielded brown liquid.

INSOLUBLE (9)
White crystals like those in (1) melting at 220°C. Few crystals melted at 135°C

SOLUBLE (5)
Ether completely removed

water

SOLUBLE (6)

Greenish tinge to the aqueous solution. On evaporation of the water obtained faintly yellow crystals, which were mixtures.

INSOLUBLE (7)

Yellowish-orange colored mixture. On heating the residue, a sublimate of colorless rods was formed at 90° C., which then melted at 144-146° C. Another part of the residue melted at 117° C.

FIG. 1

increased in size. After approximately two weeks of agitation, the colonies increased to a size that made further agitation difficult. At this time the culture became contaminated with bacteria, and the experiment was discontinued. The results for sugar analysis and pH of the medium are presented in Table II.

The data show that even under this condition of vigorous aeration, agitation, and growth of the fungus throughout the medium, the pH of

TABLE II

Effect of Agitation and Aeration on a Culture of *Chaetomium funicola* Cke.

Time of Agitation and Aeration (days)	pH of the Medium	Milligrams of Glucose Present per 1.0 cc. of Medium
0	5.01	41.75
1	5.75	40.20
5	6.55	38.30
17	6.30	34.10

the medium again moved toward neutrality, and at the same time glucose decomposition was still a slow process. Since the air that passed through the medium was in fairly large-sized bubbles, the aeration efficiency probably was not high despite the rapid passage of air through the medium. Attempts to subdivide the air into fine bubbles by passage through small blocks of wood immersed in the medium presented a difficulty as the organism grew into the pores of the wood. Similar difficulty was encountered in the above experiment in using the four-way-outlet glass tubing.

SUMMARY

Chaetomium funicola Cke. slowly converted the glucose in Czapek-Dox solution mainly to carbon dioxide and mycelium over the entire tested period of 33 days development. Products of the nonvolatile, nonacidic, and nonreducing class accumulated in the medium throughout this period. The maximum formation of these products occurred between the eighteenth and the twenty-sixth day of fungous development when approximately 35 per cent of the glucose carbon dissimilated in that period was converted into these products.

Volatile and nonvolatile acids and volatile neutral compounds accumulated in only very small amounts.

The medium gradually became alkaline with progressive development of the fungus.

Ether extraction of the concentrated residual medium yielded a mixture of brownish, liquid fatty material and crystalline organic compounds possessing different melting points.

Vigorous agitation of the culture medium by means of forced air did not hasten glucose dissimilation even though *C. funicola* grew throughout the medium.

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NEW SPECIES OF LYGUS FROM THE WESTERN UNITED STATES (HEMIPTERA, MIRIDAE)

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The writer published a revision of the genus Lygus (1917),[1] but since that time more collecting has revealed a few additional species from the western United States. Van Duzee (1918) described *Lygus abroniae* from California and later *Camptobrochis slevini* Van D. (1925). The latter has proved to be a dark color variety of *Lygus abroniae* Van D. The present paper describes four new species and a variety, some of which have turned up in economic studies by various workers.

Lygus ceanothi new species

Allied to *convexicollis* Reuter but differs in the shorter rostrum, differently shaped head, and coloration of the hemelytra.

MALE. Length 6.7 mm., width 2.8 mm. Head: width 1.14 mm., vertex .41 mm.; position subvertical, frons obsoletely striated, basal carina distinct, a triangular impression just before on vertex. Rostrum, length 2.81 mm., extending to sixth ventral segment, brownish, apex black. Antennae: first segment, length .61 mm., yellowish brown, blackish beneath; II, 1.95 mm., cylindrical, slightly more slender near base, yellowish to reddish brown, narrow base and apical one-third blackish, thickly clothed with short yellowish pubescence; III, .93 mm., black; IV, .61 mm., black.

Pronotum: length 1.27 mm., width at base 2.29 mm., moderately convex, coarsely punctate, shining, a minute yellowish hair arising from each puncture; yellowish brown, calli and two rays behind each callus, traversing disk and joining submarginal area, black; propleura pallid on lower half, a black ray beginning at top of coxal cleft and flaring toward basal margin. Scutellum moderately convex, coarsely punctate, somewhat rugulose; blackish, a median ray on apical half and a short ray each side beginning at base, pallid to yellowish.

Hemelytra elongate, embolar margins only very slightly curved, shining, punctate, clothed with short yellowish pubescence; ground color pallid, subtranslucent, clavus and corium blackish, the dark color broken by pallid spots, the central area of corium more pallid than dark but the apical area is unbroken by spots. Cuneus, length 1.38 mm., width at basal fracture .65 mm., whitish, subopaque, apex and spot at outer basal angle black. Membrane fuscous, basal half of cells, veins, and spot behind tip of cuneus, clear to dusky.

[1] New York (Cornell) Agr. Exp. Sta., Bul. 391, pp. 553-645, 1917.

Legs yellowish to brownish, femora with two subapical bands fuscous to black; tibiae yellowish, base with two dark marks, apex fuscous, spines brownish. Venter yellowish to brownish, sides with a somewhat broken brownish black stripe, more pallid just beneath. Genital claspers very similar to *convexicollis* Reut., but claw at tip of right clasper, obliquely angulate beginning at middle.

FEMALE. Length 6.7 mm., width 3 mm. Head: width 1.25 mm., vertex .52 mm. Antennae: first segment, length .58 mm.; II, 1.77 mm.; III, .90 mm.; IV, .65 mm. Pronotum: length 1.34 mm., width at base 2.55 mm. More robust than the male but very similar in coloration.

HOLOTYPE: ♂ August 20, 1925, alt. 8,500 feet, Pingree Park, Colorado (H. H. Knight); author's collection. ALLOTYPE: same data as the type. PARATYPES: 8 ♂ 8 ♀, taken with the types on *Ceanothus velutinus*. ♀ August 27, 1920, alt. 8,800 ft., Pingree Park, Colorado (H. C. Severin). IDAHO: 11 ♂ ♀, Aug. 6, 1939, Moscow Mountain, alt. 4,000 ft., Moscow (B. C. Fluke). OREGON: ♂ Aug. 7, 4,350 ft.; ♂, Aug. 29, 1930, alt. 6,600 ft., Crater Lake Park (H. A. Scullen).

Lygus ceanothi delecticus new variety

Differs from typical *ceanothi* in being less elongate and paler in color; cuneus pallid translucent, without black apex; corium only lightly infuscated apically; pronotum uniformly yellowish to brownish, a small spot behind each inner angle of calli, a larger submarginal spot on humeral angles of disk, and small ray behind top of coxal cleft, black. Scutellum with geminate black mark at middle of base, but the typical pallid marks outlined only with brownish. Femora with brownish subapical rings, base of tibia with black spot.

MALE. Length 5.8 mm., width 2.6 mm. Head: width 1.17 mm., vertex .43 mm. Rostrum, length 2.7 mm., scarcely reaching base of fourth ventral segment. Antennae: first segment, length .69 mm.; II, 2.03 mm., brownish, apical one-fourth blackish; III, .91 mm.; IV, .65 mm. Pronotum· length 1.3 mm., width at base 2.16 mm.

FEMALE. Length 6.5 mm., width 2.9 mm. Head: width 1.17 mm., vertex .47 mm. Antennae: segment I, length .65 mm.; II, 1.95 mm.; III, .95 mm.; IV, .78 mm. Pronotum: length 1.47 mm., width at base 2.47 mm. More robust than the male but very similar in coloration.

HOLOTYPE: ♂ Aug. 6, 1939, alt. 4,000 ft., Moscow Mountain, Moscow, Idaho (B. C. Fluke); author's collection. PARATYPES: 48 ♂ ♀, taken with the type. IDAHO: ♂ 3 ♀ Aug. 4, 1936, Moscow Mountain (Shull & Coon). ♀ Sept. 5, 1932, Moscow Mountain (T. A. Brindley). MONTANA: ♀ July 27, 1918, St. Regis Pass (A. L. Melander). ♂ Aug. 14, 1926, Park County, alt. 6,000 ft. (A. A. Nichol).

Lygus nigrosignatus new species

Allied to *elisus* Van D., but antennal segments shorter, body more elongate, and strongly marked with black, head with distinctive marks. MALE. Length 5.6 mm., width 2.5 mm. Head: width 1.14 mm., vertex

16 mm.; pallid or greenish, a black spot on middle of vertex, often extend-ing as a V-shaped mark on frons; a broad black line extending from tylus across middle of jugum to base of antenna, thence vertically as a narrow line to inner margin of eye, sutural margins of lora and gula also black. Rostrum, length 2.12 mm., almost but not quite attaining posterior mar-gins of hind coxae, greenish, apical segment black. Antennae: segment I, length .45 mm., black beneath, greenish above; II, 1.36 mm., greenish to yellowish brown, narrowly black at base, fuscous to blackish on apex; III, .73 mm., brownish to fuscous; IV, .58 mm., brownish black.

Pronotum: length 1.3 mm., width at base 2.18 mm.; disk somewhat more coarsely punctate than in *elisus,* ground color pallid to yellowish green, inner margins of calli, two black stripes behind each callus, the outer one invading disk of callus; and both stripes extending to join the black sub-basal margin; a small spot at middle of lateral margin of disk and mark behind top of coxal cleft, black. Scutellum pallid, middle of base with broad black mark, bifid at tip and extending to middle of disk, also a more slender black line on each side extending from middle of disk to basal angles; disk coarsely, rugulose punctate.

Hemelytra pallid translucent and marked with black; middle third of clavus except claval vein and corium except for spot on base and on middle, black; apex of embolium and extending upon outer basal angle of cuneus, and apex of cuneus, black; punctate, clothed with short, pallid recumbent pubescence. Membrane clear, veins appear white, a brownish calloused spot next to apical angle of brachium.

Legs pallid to greenish yellow, femora chiefly blackish, a pair of pallid bands before apex, also an incomplete pale band near middle; tibial spines black, knees with two black spots, one below the other; claws and tips of tarsi black. Venter blackish, sides greenish, a black longitudinal line dividing the greenish color.

FEMALE. Length 5.3 mm., width 2.46 mm. Head: width 1.17 mm., vertex .47 mm. Antennae: segment I, length .39 mm., not equal to width of vertex; II, 1.27 mm.; III, .73 mm.; IV, .58 mm. Pronotum: length 1.25 mm., width at base 2.16 mm. Very similar to the male in form and coloration.

HOLOTYPE: ♂ March 24, 1934, alt. 550 ft., Lewiston, Idaho (H. Ber-gen); author's collection. ALLOTYPE: same data as the type. PARATYPES: 2 ♂, taken with the types on alfalfa. WASHINGTON: 2 ♂ June, 1940, Wenatchee (J. B. Moore); Expt. no. 68, reported injurious to young peach fruits.

Lygus rolfsi new species

Distinguished by the elongate form, relatively long antennal seg-ments and the black color markings on a pallid to green ground color.

MALE. Length 6.7 mm., width 2.5 mm. Head: width 1.17 mm., vertex .43 mm.; basal carina strong, sinuate at middle, vertex impressed across base and curving forward on median line; frons with oblique striate lines each side of middle; color yellowish brown, tylus fuscous; gula,

bucculae, margins of lora, basal half of juga, and line extending above base of antenna, black. Rostrum, length 2.2 mm., extending to near apices of hind coxae, brownish to fuscous, apical segment and basal one largely, blackish. Antennae: segment I, length .56 mm., black, brownish to fuscous above; II, 1.82 mm., black, cylindrical, more slender at base, thickly clothed with short yellowish pubescence; III, .82 mm., black; IV, .51 mm., black.

Pronotum: length 1.34 mm., width at base 2.16 mm.; disk moderately convex, coarsely punctate, shining; color pallid to greenish yellow, calli and extending from outer angles to collar, two rays behind each callus and extending to middle of disk, basal submargin of disk and ray extending posteriorly from top of coxal cleft, black. Scutellum greenish yellow, middle of base with broad black ray extending to middle of disk, also an irregular fuscous to black line parallel with lateral margins of disk and nearly joining with the central black ray; coarsely, rugulose punctate; mesoscutum black.

Hemelytra long, embolium 2.68 mm., cuneus length 1.25 mm., width of basal fracture .60 mm.; ground color pallid subtranslucent, tinged yellowish or green, clavus except apical one-fourth, apical area of corium and irregularly bordering clavus, apical area of embolium except outer half, apex and cloud on basal half of cuneus, black. Membrane fuscous, basal half of areoles and area beyond tip of cuneus pale translucent, veins yellowish to reddish. Mesosternum and epipleura largely, black.

Legs fuscous to black, femora with two subapical bands and a broader band at slightly beyond middle, pallid; tibiae yellowish, basal marks and spines black, tarsi and tips of tibiae blackish. Venter pallid to yellowish, ventral surface and lateral line blackish.

HOLOTYPE: ♂ July 15, 1932, Yakima, Washington (A. R. Rolfs); author's collection. PARATYPE: ♂ June, 1928, Los Angeles, California (L. J. Muchmore), collected at light. This species is named in honor of the collector, Mr. A. R. Rolfs, who has favored the writer with a number of western Miridae.

Lygus shulli new species

Allied to *hesperus* Kngt., but size smaller and rostrum shorter; size and coloration suggestive of *oblineatus* Say but differs in having only one vertical black line each side of frons; scutellum yellow, with geminate black mark at middle of base; right genital clasper with terminal claw broadly arcuate.

MALE. Length 5.6 mm., width 2.7 mm. Head: width 1.17 mm., vertex .48 mm.; pale yellowish, a vertical black line (frequently obsolete) forming above base of antenna and extending to near top of eye, the apex curved inward on vertex; ventral margin of lora and sometimes a spot on juga, blackish; collum black. Rostrum, length 2.3 mm., extending very slightly behind tips of hind coxae, yellowish brown, apex black. Antennae: segment I, length .56 mm., yellowish brown, blackish beneath; II, 1.73 mm., cylindrical, tapering to more slender on basal half, yellowish brown,

narrow base and apical one-fourth fuscous to blackish; III, .86 mm., blackish; IV, .66 mm., black.

Pronotum: length 1.28 mm., width at base 2.25 mm.; yellowish to brown, outer half of callus and extending forward to sides of collar, two rays behind each callus, the outer one longer and usually extending to middle of disk, a rounded spot on basal angles of disk and sometimes the whole basal submargin, black; propleura with a strong black ray extending from top of coxal cleft to posterior margin. Mesoscutum black. Scutellum pallid to yellowish, a geminate black mark at middle of basal margin, in darkest forms extending to middle of disk; disk coarsely rugulose punctate. Dorsum punctate and shining much as in *oblineatus* Say.

Hemelytra pallid to yellowish brown, subtranslucent, middle of clavus and apical area of corium more or less fuscous to blackish; cuneus pallid, apex and spot on outer basal angle, black. Membrane pale to fumate, apical half except central area and within apices of areoles, fuscous. Legs pale yellowish, femora with a pair of fuscous to black subapical rings, anterior aspect with a series of fuscous spots; tibiae with spot in knee and incomplete ring just below, black, spines also black; tarsi yellowish, spices and claws fuscous. Venter dark brown to fuscous, each side with a longitudinal yellowish stripe. Genital claspers rather similar to those of *oblineatus* Say but the terminal claw of right clasper broadly arcuate, not angulate.

FEMALE. Length 5.2 mm., width 2.8 mm.; costal margin distinctly arcuate in outline. Head: width 1.17 mm., vertex .49 mm. Rostrum, length 2.42 mm. Antennae: first segment, length .57 mm.; II, 1.78 mm.; III, .86 mm.; IV, .66 mm. Pronotum: length 1.3 mm., width at base 2.22 mm. More robust than the male but very similar in color and pubescence.

HOLOTYPE: ♂ July 13, 1936, Mesa, Idaho (W. E. Shull), author's collection. ALLOTYPE: same data as the type. PARATYPE: 78 ♂ ♀, taken with the types. 18 ♂ ♀ July 30, 1932, Belview, Idaho (W. E. Shull), taken on smart weed (*Polygonum* sp.). ♂ 2 ♀, June, 1940, Wenatchee, Washington (J. B. Moore); (Expt. nos. 22, 85, and 99) injurious to young peach fruits.

Named in honor of Dr. W. E. Shull, University of Idaho, who has done important biological work on *Lygus elisus* Van D. and *L. hesperus* Kngt., in Idaho.

NOTES ON THE FAMILY MESOVELIIDAE (HEMIPTERA) WITH DESCRIPTIONS OF TWO NEW SPECIES

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The Mesoveliidae constitute a small family of less than two dozen known species distributed in four genera. The representatives of the family live on the surface of water in secluded coves of marshes, ponds, and lakes and in the wider reaches of streams where the water is more or less quiet and carries an abundance of floating aquatic plants. Some of the species are widely distributed. In the Western Hemisphere *Mesovelia mulsanti* White is the commonest form and occurs generally in North, South, and Insular America. A catalogue of the species then known was published by Horvath in 1929 as Fascicle II of the General Catalogue of the Hemiptera. The types of the new species described below are in the collection of the authors.

MESOVELIA AMOENA Uhler

Mesovelia amoena Uhler, Proc. Zool. Soc. London, 1894, p. 218; Jaczewski, Ann. Mus. Zool. Polonici, IX, 1930, p. 9.

Two winged females from the original type series, H. E. Summer's collection, are at hand. The species appears to have a very wide distribution and to show considerable variation in the arrangement of the light and dark color markings. Apterous and winged females are present from Trinidad, B.W.I., Oct. 27-29, 1938, C. J. Drake; Para, Brazil, H. H. Smith; Canal Zone, Panama, and Barro Colorado Island, February, 1939, C. J. Drake; and Mayaguez, Puerto Rico, June 2, 1938, M. Airlez. Several specimens, the females of which appear to be specifically inseparable from the above-mentioned examples, are present from Laurel, Mississippi, Aug. 27, 1934, H. M. Harris; Baker, Louisiana, August 30, 1934, H. M. Harris; and Waco, Texas, August 9, 1933, H. B. Mills. Males of these are also at hand, but as noted by Jaczewski, male specimens from insular America will have to be studied in order to show the relation of *M. douglasensis* Hungerford and *M. amoena* Uhler.

MESOVELIA BILA Jaczewski

Mesovelia bila Jaczewski, Ann. Mus. Zool. Polonici, VII, 1928, p. 77, pl. IV, figs. 10-13.

Two males and four females, all apterous, taken in floating aquatic plants in small, stagnant pools in an irrigation ditch about 10 miles from Tigre, Province of Buenos Aires, Argentina, Dec. 10 and 20, 1938, by C. J. Drake. One apterous female, labeled "Tigre, Buenos Aires," from

M. S. Pennington Collection. Known heretofore only from the type locality, State of Parana, Brazil. The alate form is unknown.

This species is smaller than *M. mulsanti* and lacks the spines within on the anterior femora. It is larger than the other known American members of the genus. The parameres, the two tufts of short, black spines near the hind margin of the antepenultimate segment of venter, and the fringe of spines along the posterior margin of the sixth abdominal sternite are distinctive characters.

<div align="center">Mesovelia zeteki, sp. nov.</div>

WINGED MALE: Form of *M. bila* Jaczewski, but a little smaller. Pale testaceous, the pronotum and veins of hemelytra considerably infuscated. Head testaceous, with the usual dark, setiferous spots, the interocular space slightly greater than the diameter of an eye. Eyes large, dark reddish brown, coarsely faceted. Rostrum long, reaching on base of venter; testaceous, the apical segment dark. Antennae long, segment I stout, slightly bowed and becoming slender distally, with long bristly hairs at apical third, its length slightly less than width of head through eyes (24:27); segment II a little shorter and slenderer than I; III and IV long, slender.

Pronotum distinctly broader than long (40:26), moderately narrowed anteriorly, its sides and most of posterior lobe dark fuscous, the anterior lobe mostly testaceous, the hind lobe arched, with an indistinct median pale longitudinal line. Scutellum dark, slightly larger and distinctly broader at apex than in *M. amoena* Uhler, its distal portion not so strongly impressed as in that species. Hemelytra with basal portion of clavus and most of corium white, the nervures thickened, dark fuscous. Legs moderately long, testaceous, the front femora unarmed, the long bristly hairs of tibiae and the connexivum margined with brown. Venter pale brown, the three apical segments testaceous; the penultimate segment, broadly angularly produced behind, beset along the margin with dark fuscous bristly hairs. Genital segments plump, rather closely pubescent.

Length (to apex of abdomen), 2.45 mm.; width (through humeri), 0.82 mm.

HOLOTYPE, male, old Panama City, Panama, February 10, 1939, C. J. Drake, taken near the bank in the wide reaches of a small stream. Named in honor of Mr. James Zetek who has done much to increase our knowledge of the insect fauna of the Canal Zone, Panama.

M. zeteki lacks the dense tufts of spines on the genital segments which are characteristic of *M. mulsanti* White and its relatives. It is perhaps nearest *M. bila,* however, the irregular row of spines along the margin of the penultimate segment of venter is limited to the angularly produced median portion and does not extend all the way across the section as in *M. bila,* and the tufts of spines are also not present on the

apical segment. The winged male is distinctly larger than the winged female of *M. amoena* Uhler.

Mesovelia hackeri, sp. nov.

MACROPTEROUS MALE: Shorter and distinctly slenderer than *M. hungerfordi* Hale, the genital segments without dense tufts of spines. Dark brown, the front lobe of pronotum and head yellowish brown. Head with the usual dark setiferous spots, a narrow median, double stripe paler. Interocular space faintly greater than diameter of one eye. Rostrum reaching on base of venter, segment II very long. Antennae dark brown, segment I long, rather slender, slightly bowed; II slenderer and shorter than I; III and IV very slender—proportions, I:II:III:IV= 30:22:46:47.

Pronotum moderately narrowed anteriorly, the posterior lobe arched, impressed within humeral angles. Scutellum broadly rounded apically, the disc of hind lobe impressed, its margins raised. Wings dark brown, the base of clavus paler; veins raised, very dark. Venter narrowed posteriorly, clothed with fine hairs. Genital segments plump. Legs long, slender, testaceous, the fore femora without spines; the tibiae and tarsi slightly darker in color.

LENGTH (to tip of abdomen), 2.65 mm. WIDTH (through humeri), 0.90 mm.

HOLOTYPE: male, Asharove, Australia, Feb. 3, 1931, collected by Henry Hacker, in whose honor the insect is named.

M. hackeri is the second species of the genus known from Australia. It has a distinctly shorter head than has *M. ujhelyii* Lundblad from Oceania with which it agrees in the absence of tufts of spines on the genital segment, and the setiferous points on the head are less widely separated. It is not easily confused with *M. hungerfordi* Hale, a common water-strider in Australia.

MESOVELOIDEA WILLIAMSI Hungerford

Mesoveloidea williamsi Hungerford, Bul. Brooklyn Ent. Soc., 24: 1929, p. 289; Jaczewski, Proc. Ent. Soc. Wash., 33: 1931, p. 64; Hungerford, Bul. Brooklyn Ent. Soc., 33: 1938, p. 218.

This interesting species was described from Mera, Ecuador, and has since been recorded from Cacholi, Ecuador, and from Costa Rica and Peru. Specimens are at hand from Los Amates, Guatemala, collected by Kellerman, and from Trinidad, B.W.I., Oct. 27, 1938, C. J. Drake, thus further extending the known range of the species. Only the macropterous form has been taken.

METHODS AND COMPUTATION IN FECAL ANALYSIS WITH REFERENCE TO THE RED FOX[1]

Thos G Scott[2]

Fecal analysis is adapted to studies of the feeding tendencies of some mammals. The technique is particularly useful because the many feces obtainable make possible a continuous determination of the relative quantities of foods consumed by animals in limited areas.

It may be worth while to consider those phases of the technique that seem to be weaknesses. Identification of scats and estimation of their age are problems. In most cases, however, knowledge of "sign" and acquaintance with the ecology of the locality reduce the possibilities of error. The results obtained with respect to easily digested foods or those lacking indigestible elements are uncertain, and in some instances the utility of the technique may depend on whether the animal studied consumes significant proportions of such foods. Analysts regularly identify items considered little resistant to digestion; nevertheless, some of these foods doubtless remain undetected. It appears unlikely, however, that these defects can influence the results sufficiently to obscure the principal feeding trends. Identifying foods as carrion in fecal passages is impracticable, but possibly the value attached to carrion determinations has been unduly emphasized. Such identifications are usually offered as evidence supporting a division of foods into prey killed by the feeding animal and that found dead. This segregation is desirable, but too often the evidence on which it is based is fallible. During freezing weather dead animals may remain in a good state of preservation for prolonged periods, and even under milder conditions carcasses are usually found and eaten before they have aged enough to be recognized as carrion. Further, prey may be killed by a predator and later eaten as carrion by the same individual or species.

Investigators are largely agreed in recognizing the value of fecal analysis to continuous food-habit research on biologically known areas; however, there is some disparity of opinion in respect to methods and records. In current research into the food coactions of the northern plains red fox (*Vulpes regalis*) these differences were considered, and it

was deemed advisable to appraise the relative values of various methods and computations used in fecal analysis.

<div align="center">EXPERIMENTAL PROCEDURE</div>

Through laboratory experiments it is possible to test the merits of scat analysis as a method of determining food habits. By feeding captive animals known kinds and quantities of food and subsequently collecting and examining their feces it is possible to test and improve methods of analysis and computation.

For the experiments here reported three pens and kennels similar to those used on silver fox farms were obtained in which to confine wild red foxes. Two pens (I and II) contained one fox each and the third pen (III) housed two.

Foods of known weights and kinds were given to the foxes each afternoon between five and six o'clock. Fecal passages were collected regularly each morning between eight and nine o'clock and again at feeding time. Each passage was placed in a separate bag and catalogued. Thirty-six scats were collected in Pen I, thirty-eight in Pen II, and forty-one in Pen III.

In general, the method of preparing the fecal material for analysis was as follows: Each scat was softened in warm water. The parts of diagnostic significance were separated and cleaned by washing the softened material in a sieve (twelve meshes to the inch) over a glass jar; thus, parts that occasionally passed through the sieve were caught. The remaining material was soaked in a glass beaker filled with clear water in which the heavy objects settled out of those that would float. The material was then poured portion by portion into petrie dishes where it was mechanically separated and, excepting the hair, removed with a forceps to a paper blotting towel. During this separation it was found helpful to shift the petrie dish from black to white backgrounds to make different items visible. The hair left in the dish was finally caught in the sieve and removed to the blotting towel. Identifications were based on the diagnostic parts made available by this sorting process.

<div align="center">METHODS OF ANALYSIS AND COMPUTATION</div>

The results of the analyses were variously computed as recorded in Table I. The figures shown in the table for pen I (*see* footnote 1) might have been of better quality had something been fed to bring the chicken of the last feedings through the digestive tract in normal progression. The figures, however, are accurate enough for the purposes intended. The data are discussed according to the methods of analysis used.

NUMERICAL RECORD OF INDIVIDUALS. The count of individuals was obtained from enumeration of certain predesignated parts as they appeared in the food residuum.

This is not a very satisfactory way, as in nature the predator does not always consume a complete individual in one or even in several feed-

ings. On occasion the red fox takes only a part of animals even so small as mice. The size, desirability, condition, and availability of food have a bearing on the quantity eaten as no doubt has also the extent of competition with other species for the same food.

FREQUENCY OF OCCURRENCE. Evaluation of frequency was based on the number of times the food items occurred in the series of scats examined. The two principal computations applied were determination of percentages of the passages containing a certain food and percentages of the number of occurrences. The latter was obtained through two approaches: (1) Percentages of food items calculated by groups; and (2) percentages figured for all items represented without regard to groups. These computations are self-explanatory excepting the treatment of the food items by groups. In this the food items are arranged in such a group "break-down" as to insure that among those selected for contrast none shall possess greater opportunity for scoring occurrences in a single passage than any other. It is usually thought that occurrences of foods in fecal passages are disproportionately influenced because some foods possess greater quantities of nondigestible parts than others. Chicken is frequently suggested as a food with less opportunity for scoring occurrences because of a proportionately smaller quantity of nondigestible parts. Although the proportion of chicken to rodents eaten by the foxes in the three pens varied greatly in these experiments, the figures obtained by dividing the total weight consumed by the number of passages produced indicate that the quantity of food, irrespective of nondigestible parts, required to produce a passage was nearly constant. In pen I, 66.6 ounces of food were consumed, and 36 passages were produced, an average of 1.85 ounces to the scat. For reasons already mentioned, the figures from pen I are not especially reliable. The averages for pens II and III were 2.321 and 2.434 ounces, respectively. In general, then, the available data indicate that fecal passages are produced approximately in direct proportion to the quantity of food consumed. As many recognizable foods are taken in quantities less than those required to produce a passage, however, allowance must be made through treatment of the food items in groups. For example, a red fox might eat equal quantities of chicken and mouse, the latter representing three species. As the two food groups are equal in quantity, they may be expected to occur in approximately the same number of passages, and that their frequency of appearance would then be accurate evidence of the relative quantities eaten. Should the foods be considered in the light of individual items, however, it then becomes possible to score as many as three occurrences (one for each species) to a single passage for mouse, whereas it would never be possible to score more than one occurrence to the passage for chicken. Computations without regard to grouping, then result in underemphasis of food items with limited scoring powers and, conversely, in overemphasis of items with greater capacities for scoring, as may be seen

TABLE I

Kind of Food	Food Ingested				Frequency of Occurrence					
	Individuals	Weight	Total Weight (Groups)	Total Weight (Items by Group)	Individuals	Occurrences	Total Occurrences (Groups)	Total Occurrences (Items by Group)	Total Occurrences (All Items)	Passages Containing the Food
	No	Ounces	Pctg.	Pctg.	No.	No.	Pctg.	Pctg.	Pctg.	Pctg.
AVES: *Gallus gallus**	§	47.00	70.57	70.57		30	62.50	62.50	48.39	83.33
		14.90	28.93	28.93		15	30.00	30.00	20.83	39.47
		22.00	36.15	36.15		22	37.93	37.93	31.43	53.66
RODENTIA:	25	19.60	29 43		13	18	37.50			50.00
	44	36.65	71.07		22	35	70.00			92.10
	52	38.85	63.85		10	28	62.07			68.29
Microtus sp.	13	12.95		19.45	8	18		21.09	29.03	50.00
	21	24 00		46.54	13	34		41.75	47.22	89.47
	26	25 50		41.91	4	25		32.33	35.71	60.98
Peromyscus sp.	10	5.85		8.78	5	12		14.06	19.35	33.33
	22	11.95		23.17	8	21		25.79	29.17	55.26
	23	11.90		13.56	5	13		16.81	18 57	31.71
Mus musculus	2	.80		1.20		2		2 35	3.23	5.55
	1	.70		1.36	1	2		2.46	2.77	5.26
	2	1.15		1.39	1	2		2.59	2.86	4.88
Reithrodontomys megalotis†	1	.30		.49						
Rodentia, undetermined‡ ...						8		10.34	11.43	19.51

* The data from pen I are given for each item in the top line; those from pen II in the middle line and those from pen III in the bottom line.

† Remains of this young harvest mouse were not identified in the residuum

in Table I. The desirability of group treatment is further emphasized by the impossibility of identifying by species all remains in fecal passages. This is particularly true of avian remains.

The primary food groups in these experiments were chicken and rodent, and the basic percentages were calculated from a sampling of these groups. Of Aves, chicken was the only representative; hence, further reckoning was not required. It was necessary, however, to calculate percentages for the component items in the rodent portion. Then these percentages were individually multiplied by the percentage ob-

ANALYSES OF FECAL PASSAGES

	DRY WEIGHT METHOD							VOLUMETRIC METHOD			
Weights of the Food Remains	Total Weight of the Food Remains (Groups)	Total Weight of the Food Remains (Items by Group)	Total Weight of the Food Remains (All Items)	Average Weight to the Containing Passage	Average Weight to the Containing Passage (Groups)	Average Weight to the Containing Passage (Items by Group)	Average Weight to the Containing Passage (All Items)	Measured Volume of the Food Remains	Total Volume of the Food Remains	Average Volume to the Containing Passage	Total Average Volume to the Containing Passage
Grams	Pctg.	Pctg.	Pctg.	Grams	Pctg.	Pctg.	Pctg.	c.c.	Pctg.	c.c.	Pctg.
12.992	50.96	50.96	50.96	0.4331	38.40	*38.40	35.29	27.2	43.17	0.9066	68.69
1.554	7.74	7.74	7.74	.1036	16.36	16.36	9.28	6.8	9.78	.4533	20.19
2.444	11.81	11.81	11.81	.1111	14.57	14.57	11.98	4.2	7.82	.1024	5.47
12.504	49.04			.6946	61.60			35.8	56.83	1.9888	31.31
18.533	92.26			.5295	83.64			62.7	90.22	1.7914	79.81
18.243	88.19			.6515	85.43			49.5	92.18	1.7696	94.53
10.067		39.48	39.48	.5592		43.38	45.57				
12 810		63.77	63.77	.3768		31.13	33.77				
10.702		69.51	69.51	.4281		44.78	46.14				
2.360		9.26	9.26	.1966		15.25	16.02				
4.921		24.50	24.50	.2343		19.36	20.99				
2.481		16.10	16.10	.1908		19.96	20.56				
.077		.30	.30	.0383		2.97	3.12				
.802		3.99	3.99	.4011		33.15	35.92				
.396		2.58	2.58	.1978		20.69	21.32				
4.664											

‡ Undetermined rodent evaluations indicate the possible error in the frequency of occurrence calculation.
§ A record of the number of individuals was not practicable here as the foxes would eat only a part of a chicken at a feeding.

tained for their group, thus, providing for a fair interpretation of the relative importance of each item within the group.

Another computation suggests the possibility of developing conversion factors for each food item. The figures obtained by dividing the number of occurrences into the weight fed appear to offer promise. In pen I, 47 ounces of chicken were fed; 30 passages contained chicken. This corresponds to 1.57 ounces to the occurrence. Similar calculations for pens II and III yield 1.00 and 0.99 ounces, respectively. As previously mentioned, the results in pen I, especially for chicken, were somewhat

atypical, as chicken was the last food eaten and no food was fed to bring it through the digestive tract in normal progression. Factors for all rodents are: 1.09, 1.05, and 1.39. Those for the individual genera are: *Microtus* 0.72, 0.71, and 1.02; *Peromyscus* 0.49, 0.57, and 0.92. The obvious increase in the figures calculated for the genera from pen III is of uncertain value because of the undetermined rodent food included. In general, the variation from the mean for these factors is not so great as to rule out the possibilities of their application. Tables of conversion factors for foods likely to be eaten might be prepared for each predator investigated from data gathered in extensive feeding experiments.

DRY WEIGHT. The computations applied to the dry-weight method present two approaches: Percental evaluation of the respective weights of item remains and percental calculation of the average weights of remains of each item to the containing passage.

The diagnostic parts of each passage were allowed to dry at room temperature, and were then separately weighed on an analytic balance. Segregations, as between the mixed remains of different species of mouse in the same passage, were based on percentages of the relative weights of the identifiable bones of each species, the unidentifiable bones and hair being proportioned according to those percentages.

VOLUMETRIC METHOD. Computations were based on volumetric measurements and reckoned in percentages of the total measured volume and also in percentages of the average measured volume to the containing passage. The figures were obtained by measurement of the separated material in cubic centimeters. Measurements were made in a graduated cylinder, and the material was firmly pressed down for each reading.

INTERPRETATION OF THE NUMBER OF INDIVIDUALS

Interpretations of relative numbers of individuals represented in the analysis may be attempted in predation studies on areas where prey populations have been estimated. In pen I, *Microtus* occurred in 18 passages; 13 individuals had been fed. A single occurrence then corresponded with 0.72 available individuals. Similar figures calculated from the table are: *Microtus* 0.62 and 1.04; *Peromyscus* 0.83, 1.05, and 1.77. The figures as obtained from pen III are probably overemphasized by the presence of undetermined rodent. With more extensive data it would seem possible to establish factors for estimating from the results of fecal analysis the relative numbers of individuals of prey species eaten.

DISCUSSION

It is granted that objection-proof conclusions with respect to methods of fecal analysis and computation cannot be drawn from these experiments. The information does indicate, however, the desirability of improving laboratory techniques in food-habits analysis and computa-

tion and offers suggestions as to directions in which improvement may be made.

The data, though admittedly limited, leave little doubt as to the interpretative superiority of the frequency of occurrence method of fecal analysis as contrasted with the other methods used in these experiments. The proposal for improvement of this method by computing the relative quantities through grouping of food items may be questioned; however, it may be suggested that any method of calculation that disproportions one group by subdividing another or others, as does the computation usually employed, at least merits suspicion.

Of the computations attempted with the dry-weight method, the percental evaluation of the respective weights of item remains proved most reliable. It is of considerable advantage if the laboratory method used permits keeping abreast of seasonal and unheralded feeding trends, but dry weighing was the most time-consûming and expensive method tested and cannot be recommended.

Percentages calculated from the average volume to the containing passage appeared to provide the most reliable interpretation of the volumetric method. The results are somewhat uncertain as evidenced by the results for pen III. Though not so exacting as the weight method, it demands considerable time and energy. It also limits measurement to the major food groups and thus provides no interpretation of the relative quantities of lesser items.

SUMMARY

Foods of known weights and kinds were fed to red foxes for the purpose of testing methods of analysis and computation as applied to fecal material in food-habits research.

The following methods of analysis with various computations were tested: (1) Numerical record of individuals; (2) frequency of occurrence; (3) dry weight; and (4) volumetric. The frequency of occurrence method was shown to provide for the most reliable interpretation of the relative quantities of foods consumed. A proposal was made for improvement of the results in the frequency of occurrence analysis by group treatment of the food items identified in such sequence that no group or item should possess greater potential for scoring occurrences in the same passage than those with which it is contrasted. The possibility of developing conversion factors for each food group or item for application to frequency indices in determining relative amounts was suggested. A technique for calculating the relative numbers of individuals represented in the frequency of occurrence analysis was discussed.

THE USE OF IOWA CLAYS[1] FOR THE CLARIFICATION
OF SORGO SIRUP[2]

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INTRODUCTION

The production of sorgo sirup in the United States decreased from a high of 49½ million gallons annually in 1920 to a low of 9 million gallons in 1930. Since that time an average of about 14 million gallons has been made per year (16). Although the sirup is not used as universally as formerly, it still enjoys a considerable demand as a table sirup. It has a mild acid flavor that is characteristic and unique.

The making of sorgo sirup has in the past been more of an art than a science. Nearly every community at one time had its sirup maker, as evidenced by the amount of equipment still available on many farms. For some time farm production of sirup has declined and factory production has increased. Recent trends toward diversified farming, however, and reductions in surplus crops acreage, have turned farmers' attention to the growing of small amounts of other crops which may be used to reduce living expenses or as cash crops. Sorgo, although classed as a soil-depleting crop, satisfies both of these requirements in that the sirup may be used by the farmer or sold. A yield of about 100 gallons of sirup per acre may be expected in addition to 20 to 30 bushels of seed.

The farmer who wishes to make sorgo sirup encounters two difficulties. First, he must resurrect what is, in many places, almost a forgotten art, and secondly, his product must be sold to a critical buying public that has become accustomed to the clear, light-colored corn and maple table sirups and to the clear sorgo sirup now being produced on a factory scale. The heavy, dark-brown, viscous sirup often made is viewed with suspicion.

The object of the present study was to ascertain the essential details for the production by the Iowa farmer of a clear, high-grade, commercially acceptable sirup which could successfully compete with factory-made products.

It was found that the use of certain types of clay for clarification of sorgo juice resulted in a very fine grade of sirup. Several clays were examined to determine the characteristics necessary for this purpose and to ascertain the optimum conditions for preparing sirup on a farm scale

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[1] The word "clay" is used in a general sense to include materials also known as loess and gumbotil.

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by this method. Under the same conditions when the older method was compared with the clay clarification method, it was found that the latter method was economically feasible. Although the loss when using clay, was a little greater, the grade of sirup was much improved, and the capacity of the mill was approximately doubled.

METHODS OF PRODUCING SORGO SIRUPS

The cultivation, growing, harvesting, and milling of sorgo cane are not within the scope of this paper. Briefly, the cane is grown and culti- vated about like corn and harvested in September before freezing weather. Leaves are stripped from the cane, and the seed head and two or three top joints are removed (to lessen acidity (15)). The cane is cut and brought to the cane mill where it is pressed between rollers and the sorgo juice, after being strained through a coarse screen, is then ready to be made into sirup. It should be noted that juice which stands longer than a few hours is very likely to spoil by fermentation, a fact which greatly increases the difficulty of research. A poor tasting sirup may result from cane which is raised on land highly fertilized by manure. Occasionally a red juice is obtained which makes a poor grade of blood- red sirup. The cause is not known but further work on this subject is being carried on at Iowa State College.

The following four general methods of preparing the sirup are used:

1. Factory process: The sorgo juice is clarified by absorbents and heat, and filtered through filter presses. A complete description of factory practice has been given by Bartling (1). The process is not suitable for farm use. The sirup is clear and dark yellow in color with a mild taste since the acidity is controlled. The sirup is generally thinner than farm sorgo sirup and thus can be sold for a lower price.

2. Old process: The farm method used for many years is to coagulate the green material in the juice by boiling and then skimming it off constantly. The process is tedious and laborious, and the product is usually dark brown due partly to the long-continued heating (18).

3. U.S.D.A. process: The method recommended by the United States Department of Agriculture as described in Farmers' Bulletin No. 1791 (16) is essentially an elaboration of the old process mentioned above. For juices that contain starch, which will cause the sirup to "jelly," it is recommended that the juice be partially evaporated and then malt extract added to the semi-sirup to convert the starch. The mixture is allowed to stand over night and the evaporation continued.

4. Clay clarification process: This process involves the addition of a small amount of clay to the cold juice which causes the green material to coagulate. The clarified juice is then decanted from the clay and may be boiled down rapidly with a minimum of skimming. It has been found that this process is being used successfully on some farms and that it gives the best sirup of any examined, including the factory-made pro-

duct. This process was, therefore, studied at considerable length with the object of determining the details of the clay clarification method, comparing its yield with that of the older process on an economic basis, and of examining the clay varieties which gave satisfactory clarification. A preliminary popular report of some observations on this subject has been published by the authors (8).

HISTORICAL AND THEORETICAL

The use of clay for clarification of sorgo juice is rare but not new. Some writers (3) disparage the use of clay for this purpose. In 1893 the United States Department of Agriculture (17) described the examination of 200 clays for this use and stated that clay is very good for this purpose. Theoretically clay should serve very well as a coagulant. The impurities present in the juice are probably nitrogenous and carbohydrate materials such as chlorophyl, starch etc., all in colloidal suspension. The reaction of the juice is slightly acid. The investigations of Demolon and Barbier (5) show that humic colloids are strongly adsorbed by clay when the solution is acid. Mattson (10) found that the isoelectric point of proteins was lowered by the addition of bentonite, perhaps due to the formation of a non-ionized complex with the proteins. Meyers, too, has observed (12) that the tendency of organic colloids to combine with inorganic soil colloids was greater in acid suspensions and suggested polar adsorption as the probable reaction involved. Ensminger and Giesking (6) have observed that proteins are more strongly adsorbed in suspensions of high hydrogen-ion concentrations than in suspensions of low hydrogen-ion concentrations. They suggest that the adsorption of proteins as cations is partly responsible for their combinations with montmorillonite materials. Considering the work of these men and others as well as the appearance of the clay and juice after clarification, it seems probable that most of the organic colloids present are adsorbed by the clay particles and that these particles then coagulate with each other and sink through the juice thus occluding and sweeping out other particles. When the clay is stirred with the juice, an appreciable interval occurs during which no reaction seems to be taking place. Then the suspension suddenly "breaks," and the particles settle rapidly. Clarification is sufficiently complete so that the boiling off may be done in a relatively short time, with comparatively little skimming.

A detailed investigation of the theoretical factors influencing the clay clarification is extremely difficult. The mechanism of coagulation of clays by a consideration of the surface condition of the particles and the adsorbed ions which are present has been dealt with by Meyer (11) among others. It has been found (4) that clays which are apparently identical physically but differ in the nature of their clay mineral content will act very differently. Furthermore, even though the clays used are identical, a second difficulty is introduced by the fact that fresh sorgo

juice must be used if results are to be identical. Fresh juice may be obtained only during the rather short sorgo season. The juice decomposes very rapidly on standing even though toluene, chloroform, and low temperature storage are used to preserve it, the colloids coagulate and settle out to some extent, and this makes the juice very easy to clarify. Small-scale work is difficult to evaluate with regard to yield due to errors introduced in attempting to boil down and skim small batches of juice.

Practical tests were therefore developed by which different clays could be tested against each other, the various factors observed, and then large-scale runs made to determine the economic factors involved. Two apparently identical clays which gave considerably different results with regard to clarification were examined exhaustively to determine the causes of this difference in behavior.

EXPERIMENTAL WORK

ACIDITY. The pH of fresh juice was found to be approximately 5. The acidities of the fresh juice, the clay clarified juice, and the final sirup were found to be practically identical.

PRECIPITATING AGENTS. It was found that 1 per cent sodium chloride, 1 per cent aluminum sulfate, 1 per cent sodium silicate and 2 per cent freshly precipitated silicic acid, added to separate samples of juice were not suitable colloid precipitants. When 0.6 gram of $Al_2(SO_4)_3$ was added to 100 cc. of juice and neutralized with the equivalent amount of Na_2CO_3 the resulting juice was water-white, but the precipitate took a long time to settle out.

Cider is often clarified by use of a gelatin-tannin mixture (7). It was found that by varying the ratio of gelatin to tannin according to acidity, the juice could be satisfactorily clarified, but the resulting sirups had bad color and taste. "Pectinol" (9) was found to be useless for the clarification of the juice.

COMPARISON OF CLAYS

Preliminary work established the fact that "8 per cent" of clay (24 grams of dry pulverized clay per 300 cc. of juice) gave optimum results with a clay ("Hartford Clay") now being used successfully for commercial sirup production. The clay could be reused twice satisfactorily. However, under working conditions in warm weather, the clay may be used only twice in order to avoid spoilage of the juice. The following procedure was then adopted to determine empirically the usefulness of clays in general for juice clarification.

Twenty-four grams of pulverized clay were added to 300 cc. of fresh juice in a 500 cc. stoppered flask and immediately shaken to wet the clay. The flask was then shaken for 20 minutes and the contents, clay and all, were poured into a graduated cylinder. The rate and amount of settling and the volume of recoverable juice were noted and the clear

TABLE I

COMPARISON OF VARIOUS CLARIFYING AGENTS ON SORGO JUICE

Sample No	Type of Clarifying Agent Used	Rate of Settling	Color of Juice	Sirup Produced	Rating
1	Clay (Hartford, Iowa)	Very fast	Clear grey	Excellent	Excellent
2	Sandy loess (Redfield, Iowa)	Very fast	Very green	Discarded	Bad
3	Sandy Clay (Lehigh, Iowa)	Very fast	Very green	Discarded	Bad
4	Kaolin (Columbia, S. C.)	Slow	White	Excellent	Good
5	Bauxite Waste from Aluminum Co., (St. Louis, Mo.)	Did not settle	Red	Discarded	Bad
6	Mortar mix (Sheffield, Iowa)	Fast	Grey	Very good	Very good
7	Mortar mix (Adel, Iowa)	Fast	Grey	Very good	Very good
8	Brick Clay (Auburn, Iowa)	Fast	Green	Discarded	Bad
9	Crown Ball Clay (England)	Moderate	Clear grey	Very good	Good
10	Ball Clay (Kentucky)	Slow	Greenish grey	Poor	Poor
11	Vollendar Clay	Slow	Clear grey	Very good	Good
12	Kaolinite (Florida)	Slow	Clear grey	Excellent	Good
13	Limey Shale (Mason City, Iowa)	Slow	Clear grey	Excellent	Good
14	Shale (Des Moines, Iowa)	Fast	Clear grey	Very good	Very good
15	Bentonite ("Volclay")	Very slow	Clear grey	Good	Bad
16	Clay (Lynnville, Iowa)	Very fast	Clear grey	Excellent	Excellent
17	"No. 1263"*	Fast	Green	Poor	Poor
18	"No. 1316"*	Fast	Clear grey	Very good	Very good

* These two clays are described in considerable detail in the text

liquid siphoned off. The juice was then boiled down in a long narrow pan heated only at one end.

"Skimmings" were removed from the cold end by means of a fine wire screen. The juice was stirred near the end of the process to prevent scorching and was removed from the heat when the boiling point of the sirup was 111°C. The sirup was strained through the skimming screen and the turbidity, color, and taste observed. Although no quantitative results were possible, a satisfactory comparison could be made of the clays.

The results, using some Iowa clays and a few other available materials, are given in Table I.

From Table I it is evident that a number of clays are available for sirup clarification. Such clays can be obtained and their clarifying power determined by means of a practical test using fresh juice. The clay, of course, should give no objectionable taste to the juice. This may be ascertained by shaking the clay with water, allowing the clay to settle, and tasting.

CHARACTERIZATION OF CLAYS

A very complete investigation was made of the clay minerals of two clays numbered "1263" and "1316" in Table I. Clay 1263 was from Marshall County, Iowa, and clay 1316 was from Cass and Pottawattamie counties, Iowa. Both were loess materials and very similar in appearance and physical properties. However, as shown in Table I, practical tests indicated that their reactions with sorgo juice were very different, and it was felt that a close examination of the clay minerals present might show why one could be used successfully for juice clarification and the other could not. The two clays were road subgrade materials which, although they could not be distinguished by the usual standard highway laboratory tests, showed considerable difference in behavior when used for road foundations, the sample 1263 being unsatisfactory (4).

The loess samples were put into suspension and the material less than one micron in diameter separated by sedimentation methods. The coarser material left was nearly all smaller than 270 mesh size. The fine material of 0.005 mm. or less amounted to 25 to 28 per cent of the total, and the material of 0.001 mm. in diameter (1 micron) amounted to approximately 15 per cent in each case. This fine material was then fractionated with a supercentrifuge, according to the methods of Bray, Grim, and Kerr (2), into three fractions: the coarse colloid, particles

TABLE II
RESUMÉ OF MECHANICAL ANALYSES BY SEDIMENTATION AND CENTRIFUGE

	SAMPLE No 1263		SAMPLE No 1316	
	Original Wt. 1,500 gms	Percentage	Original Wt 1,500 gms	Percentage
Residue	1,071.81	71.45	1,176.35	78.43
Coarse colloid	105.08	7 00	113.40	7.56
Fine colloid	37.67	2.51	46.36	3.09
Superfine	104.20	6 94	91.09	6.07
Total colloids	246.95	16 45	250.85	16.72
Total colloids and residue ..	1,318.76	87.90	1,427.20	95.15
Lost in leaching and handling	181.24	12 08	72 80	4.85

approximately 1 to 0.1 micron in diameter; the fine colloid, 0.1 to 0.06 micron; and the superfine colloid, all those less than 0.06 micron in diameter. These fractions were then studied microscopically, chemically, and by X-ray analysis.

A fractionation or mechanical analysis of material of this nature is essential mainly for three reasons:

1. To obtain an approximation of the size grade distribution of particles.

2. To separate the active or colloidal fraction from the relatively inert or non-colloidal fraction.

3. To facilitate microscopic study, X-ray analysis, chemical analysis,

TABLE III
X-RAY DATA*—COLLOID FRACTIONS

	COARSE		FINE		SUPERFINE		d Values	
	1263	1316	1263	1316	1263	1316	A	
1		M		?	?	S	13.64	Montmorillonite
2	M	W	M	W	M	W	9.85	Illite
3	M	W	W	W	?		7.25	Kaolinite, Illite
4	WW	WW	W	W	W	W	4.94	β Quartz
5	S	S	S	S	S	S	4.49	Clay**
6	S	S	WW	WW			4.27	Quartz
7	M		W	W			3 55	Kaolinite, Quartz
8	SS	SS	S	S			3.32	Illite
9	M	M					3.00	Calcite
10	S	S	S	S	S	S	2 59	Clay**
11	M	S					2.45	Quartz
12	M	M					2.28	Quartz
13	W	W					2.24	Quartz
14	S	S					2.12	Quartz
15	M	M	WW	WW			1.98	Illite, Quartz
16	S	S					1.81	Quartz
17	W	W	W	W	W	W	1.66	Clay**, Quartz
18	S	S					1.53	Quartz
19	S	S	S	S	S	S	1.49	Clay**
20	S	S					1.37	Quartz

* Observed intensities:
  S.S. = Very strong
  S. = Strong
  M. = Medium
  W. = Weak
  W.W. = Very weak
** Not diagnostic of any particular clay mineral.

and base-exchange determinations by attempting to secure separates of relatively pure minerals.

The results of mechanical analyses by sedimentåtion and centrifuge are shown in Table II. The constituent clay minerals of the colloid fraction are best shown by the X-ray data in Table III which are in close agreement with the information gained through microscopic and chemical analysis; that is, they show that the principal difference between the two clays is the greater proportion of the clay mineral, montmorillonite, in sample 1316 than in sample 1263. The total base-exchange capacities of each of the colloidal fractions of the two samples are given in Table IV. The great difference in the base-exchange capacity between the colloidal and the noncolloidal fractions of both samples is striking. It is especially interesting to note the difference in the values of the superfine

TABLE IV

TOTAL BASE-EXCHANGE CAPACITY EXPRESSED AS MILLIEQUIVALENTS PER 100 GRAMS OF COLLOID

	Residue	Coarse Colloid	Fine Colloid	Superfine Colloid
Sample 1263	8 70	48 30	69 22	79 05
Sample 1316	13 20	45 31	65 98	95 24

colloid fractions of the two samples, sample 1316 having a noticeably higher value than 1263, due to the greater amount of montmorillonite present.

Inasmuch as the only significant difference between the two materials studied was the amount of montmorillonite present, and as montmorillonite is the significant clay mineral responsible for the adsorptive properties of a clay, it was concluded that the difference in the characteristic behavior of the two materials is due to this clay mineral content. This conclusion is in accord with the known facts of other high-grade commercial clarifying agents that are on the market. These agents, such as fuller's earth and bentonite clays, all contain the mineral montmorillonite which is believed to be responsible for the clarifying action.

However, for practical purposes, particle size also enters into the action. It was found that while the use of commercial bentonite, made up almost entirely of montmorillonite, resulted in a clear juice, the time required for gravity separation was so long as to be impractical. Apparently enough material of a particle size larger than that of the typical clay mineral must be present for fairly rapid settling. These larger particles probably act as nuclei around which the adsorptive clay mineral, montmorillonite, may cling. The importance of distribution of particle size as well as the distribution of the clay mineral species has been recognized in other fields, for example in paper coating clays (13),

and their influence in clarifying sorgo juice adds another interesting application.

DESCRIPTION OF CLAY CLARIFICATION OF SORGO JUICE

Following is a description of the apparatus and process used, on a farm scale, in determining the loss of sirup by this method as compared with that lost when using the older process. The process is given in considerable detail so that the information may be of value to anyone desiring to use this procedure:

Sorgo cane juice is run from the mill through a screen into one of two wooden settling troughs 3½ feet wide, 7¾ feet long and 1 foot deep. (Deeper and narrower troughs would be advantageous since this would facilitate decanting the juice from the clay.) About 115 gallons of juice are run into the tank, and then the flow is diverted to the second tank. About 6-9 gallons (8 per cent of dry clay based on total weight of juice used) of thoroughly wet clay is then added to the juice and the muddy mixture is stirred for 10 to 20 minutes until it suddenly "breaks" and the coagulated clay mixture sinks to the bottom of the tank. The juice is allowed to settle for 20-30 minutes and is then decanted through a cloth bag by means of a swing pipe. The clay is reused on a second batch of juice. After the second use, a little water may be added to dilute the clay mixture and the liquid obtained added to the next tank-full. The clay is then discarded. The strained and clarified juice is now a light grey color and is run by gravity into the boiling pans. The pans used were of galvanized iron, 8½ feet long, 3 feet wide, and 13 inches deep. An unheated "dead end" 2¼ feet long and 10 inches deep was at one end of the tank. (The pans would be easier to use if they were 15 inches deep and if a longer dead end were provided.) The juice was evaporated by heat from steam coils made from 1-inch galvanized pipes, being boiled down to sirup in about 1 to 1.5 hours. A small amount of coagulated material ("skimmings") will collect at the cool end and should be skimmed off occasionally. When about two-thirds of the juice has boiled away, rapid boiling will produce a large amount of foam. This foam is easily dissipated by the addition of a small spoonful of lard. (Lard will *not* break the foam produced by juice which has not been clarified by the clay treatment.) When the boiling point has risen to 232°F. (111°C.), the sirup is done and may be run through a screen (about 40 mesh) into a cooling tank. Any clay sediment that is not strained out of the juice will ordinarily collect in the dead end of the evaporating pans.

The sirup obtained by this method is a light yellow liquid, clearer and lighter in color than any commercial product examined.

Sirup was also made by the old skimming process using juice from the same batch of cane and using the same equipment. Preliminary runs had shown that the loss was approximately 5 to 7 per cent greater by

the clay clarification method than by the older process but that this was more than compensated for by the improved quality of the sirup and the saving of time and labor.

The following is typical of the results obtained. The juice and sirup were carefully weighed and results were based on pounds of sugar, to compensate for unavoidable differences in specific gravities of the solutions. Samples were taken after each operation and immediately boiled to avoid fermentation. Allowance was made for any loss in weight dur-

TABLE V

COMPARISON OF SORGO SIRUP YIELD BY CLAY CLARIFICATION METHOD AND BY OLD SKIMMING METHOD

| SAMPLES TAKEN | OLD PROCESS | | CLAY CLARIFICATION PROCESS | | | |
| | | | RUN I | | RUN II | |
	Weight in Pounds	Sugar Content Pounds	Weight in Pounds	Sugar Content Pounds	Weight in Pounds	Sugar Content Pounds
Raw juice	668.6*	105.2	967.3†	152.0	892.1‡	139.2
Clay used (dry basis)	....	....	72.5	....	72.5**	....
Juice and clay left after clarification ..	....	....	327.8	34.2	324.4	43.2
Juice recovered from sludge for next run	....	....	327.8	34.2	56.4	9.3
Clay sludge discarded	....	....	none	none	268.0	33.8
Skimmings lost	20.5	6.2	3.6	1.1	4.0	1.8
Sirup yield	142.8	100.4§	167.1	117.5‖	179.8	126.4¶
Net loss	....	6.2	....	35.3	....	1.4
Percentage loss	5.9		23.2††		1.0	
Average loss per run	5.9			12.1		
Average boiling time per gallon of juice in minutes	1.3			0.76		

* 74.7 gallons of juice.
† 108.5 " " "
‡ 99.5 " " "
   12 3   sirup (Sp. gr. 1.39)
   14.3 " "
¶ 15.5 " "
** The clay of run I was re-used.
†† Includes the loss put over into the second run.

ing boiling. The samples were then analysed for total sugar by the Shaffer and Hartmann method (14), and results were calculated to the same total sugar basis. Results of a typical run are given in Table V.

The percentage loss was figured on the actual amount of sugar lost rather than upon the sirup recovered because this could be measured more accurately. From the data presented in Table V, it is evident that about 6 to 7 per cent more of the total yield of sirup will be lost in the

clay method as compared to the other method. This loss is a variable in that it is dependent upon the juice left in the sludge at the end of the run. As this sludge is re-used in succeeding runs, the loss becomes greatly reduced. Furthermore, more than 80 per cent of the feed value of the skimmings will be lost. To counterbalance this, however, is the great saving in time and labor and the increased value of the product. No allowance has been made in Table V for recovery of juice by washing the clay. It was found that after washing the clay, it was possible to recover an amount of liquid exactly equal to the volume of wash water used. Since the amount of sorgo juice lost in the clay is approximately equal to 24 gallons, washing with 24 gallons of water would give a 50 per cent mixture of juice and water, thus reducing the loss appreciably. The increased cost of evaporating the excess water would, of course, counterbalance this to some extent. Probably washing with 12 gallons of water, which should recover about eight gallons of juice, would be profitable and would reduce the average loss per run almost 4 per cent to about 8 per cent, or only 2 per cent more than the older method.

One of the greatest advantages of the clay process over the older process is the great saving of time. When batches are run as indicated in Table V, the time of boiling is cut almost in half. Also, larger batches may be run because foaming is greatly reduced when boiling is rapid. Since the boiling-down period is the "bottleneck" of the process as carried out on the farm, this saving is a very important factor in favor of the clay process. The shortness of the sorgo season necessarily limits investment to very simple, rather ιc ν-cost equipment, since such apparatus must stand idle a great part of the year. The acreage of sorgo to be planted must be rather carefully considered in order to be able to process all of the crop before freezing weather arrives. In such cases the clay-clarification method shows its worth, since the capacity of a plant is approximately doubled because of the rapidity with which the clarified juice may be boiled down. The tedious labor of continuous skimming is avoided, and, although the sirup must be tended closely, the workman is free to do a considerable amount of incidental work around the sirup house. Finally, the product is so much improved in appearance and in flavor that the increased selling price which it should command would easily make up for the 6 per cent loss.

CONCLUSIONS AND SUMMARY

It has been found that Iowa clays may be used to clarify sorgo juice for the production of sorgo sirup on the farm. Such sirup is of equal or better quality, with regard to taste, clarity, and color, as compared to that produced by other methods. A description of the process and a practical method of testing clays for their clarifying power is given. An exhaustive examination of two clays of very similar appearance, but which had a considerably different effect upon the clarification of sorgo

juice, showed that the principal difference was that the clarifying agent contained the clay mineral, montmorillonite. Examination of a number of clays indicated that particle size was also an important factor in the practical use of the clay for clarification since the clay must settle fairly rapidly after clarification. Clays suitable for this use are fairly widely scattered in Iowa.

It was found that although the yield of sirup is about 6 per cent less by this method, the saving in time and labor and the improved quality of the product amply compensate for this loss.

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THE FRUCTOSAN CONTENT OF SOME GRASSES ADAPTED TO IOWA[1]

A preliminary survey

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The presence of fructose polysaccharides in members of the Gramineae has been reported at various times and names such as graminin, triticin, etc., have been given to these inulin-like carbohydrates (2). Archbold (1) has recently reviewed the literature in this field. Most attention has perhaps been given to the fructosans of cereals, such as barley and wheat, in the plants of which polysaccharides of this nature appear as a transitory reserve in amounts which rarely exceed 2 to 3 per cent. Certain grasses under the climatic conditions of England have been shown to contain, at an intermediate stage of growth, much larger amounts (4, 5). In rye grass (Western wolths) a maximum of over 30 per cent of the dry weight of the aerial part of the plant was found at the heading stage, equivalent to about 400 lb. fructosan per acre. As much as 43 per cent was present in the first internode above the surface at this time. As the plant matured, however, the fructosan content was found to fall rapidly, probably by conversion to structural constituents. Finally, at maturity, the fructosan content of rye grass was only 3.2 per cent. Young second growth rye grass did not contain as high a content of this polysaccharide as first growth material of approximately equal nitrogen content and stage of growth. A diminishing fructosan content was found in orchard grass or cocksfoot (*Dactylis glomerata*) though the peak attained was only 11 per cent, this is the youngest sample (6). The mature grass in this case, however, contained as much as 8 per cent fructosan. Nitrogen fertilization had the result of reducing fructosan accumulation on a percentage basis. Observations of a similar nature were made in the U.S.S.R. by Morosov (3) on *Bromus inermis, Lolium perenne,* and *Festuca pratensis.*

The survey reported herein was made in the summer of 1939 with the intention of ascertaining whether any grasses adapted to Iowa conditions contain at some stage of growth sufficient fructosan to justify the study of its commercial extraction to supplement the inulin process as a source of fructose. With this aim in view it was only justifiable to examine grass samples taken at a stage when the dry weight yields per acre were appreciable. Inasmuch as previous work had pointed to the time of heading as

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the period at which fructosan might be expected to reach its highest level, most of the grasses were first sampled as they neared this stage. Second cuts were taken in some cases.

METHODS

Samples. The grass samples were collected at the Agronomy Farm, Ames, and put in a hot air drier within two or three hours to inactivate enzymes. Grinding to about 50 mesh was carried out in a Christie and Norris mill.

The following grass species were analyzed: Brome grass (*Bromus inermis*), Kentucky bluegrass (*Poa pratensis*), Reed canary grass (*Phalaris arundinacea*), Orchard grass (*Dactylis glomerata*), Domestic (western) ryegrass (*Lolium multiflorum*), and Sudan grass (*Sorghum vulgare* var. *sudanense.*

Fructosan determination. The procedure followed involves extraction, hydrolysis, and determination of reducing power after removal of aldoses by oxidation to the corresponding aldonic acids. This may only be considered a satisfactory method in the absence of sucrose, the fructose component of which would be determined as fructosan.

A 3-gram sample was extracted with 100 ml. water in a boiling water bath for 30 minutes. The residue after filtration was thoroughly washed with hot water and the filtrate and washings combined in a 250-ml. volumetric flask, to which was added 3 ml. saturated neutral lead acetate solution before making up to volume. Fifty ml. clear supernatant was pipetted into a 100-ml. flask, and 1 ml. 10 per cent disodium phosphate added to precipitate the excess lead. Full precipitation occurred on neutralization with sodium hydroxide, a drop of phenolphthalein being added to determine the end point. After filtration 50 ml. were hydrolyzed under reflux by boiling with 1 g. dry oxalic acid. Partial neutralization was effected by addition of precipitated calcium carbonate. Ten ml. of the cold filtrate were placed in a 25-ml. volumetric flask, and neutralization was completed with sodium hydroxide if necessary. To this was added 2 ml. 0.1 N iodine (put into solution with 34 g. potassium iodide per liter) and 1 ml. 1 per cent sodium hydroxide. The flask was shaken, stoppered, and put in a refrigerator, not over 10°, for 2 hours. After acidification by addition of 1 ml. 0.3 N sulfuric acid, the excess iodine was removed by titration with sodium sulfite. The concentration of the sulfite is immaterial, except that not over 7-8 ml. should be needed. A burette with a fine jet was used and one drop dilute starch solution added to obtain the end point. After completion of this titration the excess sulfuric acid present was neutralized by 0.1 N. sodium hydroxide, and the volume made up to 25 ml. Five ml. of this solution were heated with 5 ml. of the Shaffer-Somogyi copper reagent in the usual way (7). The sugar content was read off on a curve previously obtained. It is to be noted that the reducing value of fructose is influenced by the presence of iodide, and unless this be taken into account, appreciable error may be introduced. The final sugar titrations were run

in triplicate, one being used to establish quickly the approximate endpoint and the second and third to determine it accurately.

The dilution system indicated above (1:250) is satisfactory for low fructosan concentrations, but may be varied in one or more places if larger amounts are present. Dilution after removal of the excess iodine remaining from the hypoiodite oxidation may be to 50 ml. or 100 ml. if necessary,

TABLE I

REDUCING POWER OF FRUCTOSE IN THE PRESENCE OF IODIDE, USING SHAFFER-SOMOGYI REAGENT 50 CONTAINING 150 ML. 0.1N IODATE PER LITER

| FRUCTOSE MGM. IN 5 ML. | TITRATION VALUE IN ML 0 005 N THIOSULFATE | | | |
	Without Added Iodide	0 4% Iodide (25 ml Volume)	0 2% Iodide (50 ml Volume)	0.1% Iodide (100 ml Volume)
1.194	10.55	9.95	....	....
1.160	10.23	9.60	9 83	9.95
0.910	7.98	7.35	7.58	7.72
0.575	5.02	4.38	4.65	4.83
0.287	2.45	1.95	2.10	2.30
0.132	1.18	0.93	....	....

but in such an event a different curve must be used for ascertaining the sugar present because of the reduced iodide content. Data for constructing the necessary curves are given in Table I.

RESULTS AND CONCLUSIONS

The fructosan contents of the various grass samples are given in Table I. Fructosan was found in all species, but the amounts even in young growth were relatively small, in most cases being of the order of 2-3 per cent. The highest content found was 7.6 per cent in first growth sudan grass at the time of blooming. For this reason second growth sudan was sampled at intervals of three to four days, but at no stage was this figure exceeded, the peak of 6.6 per cent being reached before heading.

On the basis of this survey the conclusion must be reached that the fructosan content of grass species adapted to Iowa conditions is not sufficiently high to justify the hope that they might serve as a supplementary source for the preparation of fructose.

The results do, however, raise certain questions concerning the physiology of grasses, and their carbohydrate transformations of which little is known. The fructosans appear to be ubiquitous reserves in grasses, but perhaps only of a transitory nature. Their accumulation and disappearance may be related to growth conditions since there was, for example, a greater change in percentage content in sudan grass samples taken but two or three days apart than could be accounted for by changes in total dry weight. Of the two species previously studied in England, namely ryegrass and orchard grass, the former is not well suited to Iowa conditions, and the latter is not commonly grown. Accordingly, only three

TABLE II
FRUCTOSANS IN IOWA GRASSES, 1939, EXPRESSED ON THE OVEN DRY BASIS

Grass Species	Date Cut	Plant Height Inches	Notation	Fructosan Percentage*
SUDAN	May 29	6	Young spring growth.	3.67
(Sorghum	June 9	16	Leafy growth.	5.18
vulgare	June 20	27	Leafy growth, no heads.	2.33
var.	June 26	33	Heads emerging.	5.41
sudanense)	July 3	36	In bloom.	7.60
	June 20	21	Second growth since May 29 harvest.	2.58
	July 3	8	Second growth since June 26 harvest.	4.00
	July 11	16	Second growth since June 26 harvest; leafy.	5.34
	July 14	18	Second growth since June 26 harvest; leafy.	6.62
	July 19	27	Second growth since June 26 harvest; leafy.	4.08
	July 21	36	Second growth since June 26, no heads	4.14
	July 24	38	Second growth since June 26; heads beginning to emerge.	3.29
	July 26	40	Second growth since June 26; many plants in head.	2.50
	July 29	43	Second growth since June 26; plants starting to bloom.	2.77
	July 31	44	Second growth since June 26; plants in bloom.	4.02
	Aug. 2	51	Second growth since June 26; plants in full bloom	4.07
	Aug. 5	51	Second growth since June 26; late bloom	2.79
SMOOTH BROME-GRASS				
(Bromus	May 2	11	Fresh young growth.	2.89
inermis)	May 29	16	Plants starting to bloom.	1.69
	May 29	14	Second growth since May 2 harvest.	1.91
KENTUCKY BLUEGRASS				
(Poa	May 2	8	Young leafy growth.	3.57
pratensis)	May 29	4	Second growth since May 2 harvest.	3.02
	June 9	4	Second growth since May 15 harvest.	3.82
	June 20	8	Second growth since May 15 harvest.	2.12
	July 11	11	Second growth since June 1 harvest.	3.76
ORCHARD GRASS				
(Dactylis glomerata)	May 29	27	Plants heading.	2.54
REED CANARY				
(Phalaris	May 2	9	Fresh young growth.	2.55
arundinacea)	May 29	11	Leafy growth; no heads.	3.01
	June 9	14	Many heads emerging.	3.11
	May 29	8	Second growth since May 2 harvest.	2.38
DOMESTIC RYEGRASS				
(Lolium	June 9	5	Young growth; leafy.	3.60
multi-	June 20	8	Heads starting to emerge.	2.93
florum)				

* Expressed as total fructose after hydrolysis.

samples of them in all were taken. The fructosan content of these was far lower than occurs at approximately the same stage under English conditions. Similarly, the samples of brome grass analyzed by Morosov (3) contained considerably more fructosan than the three samples reported herein.

SUMMARY

A survey was made of the fructosan content of some of the more important grass species adapted to Iowa conditions in order to ascertain whether any of these might serve as a source for the preparation of fructose. In almost all samples the content was of the order of 2 to 4 per cent, which would not be sufficiently high for this purpose. The highest amount found, 7.6 per cent, was in sudan grass at the blooming stage.

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STUDIES ON THE MYXOBACTERIA[1]

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1. Distribution in Central Iowa With Description of a New Species

As a consequence of the development of satisfactory techniques for the isolation of myxobacteria, the Krzemieniewskis (1926) found that many species previously thought to be more or less obligate coprophiles could be cultivated from the soil. In much of their work they relied solely on soil samples and were able not only to re-isolate many species that had been previously found only on dung, but to discover several new species. In undertaking the present work a modification of the Krzemieniewski isolation technique was employed.

METHODS OF ISOLATION

Samples of soil, and of dung of various kinds, from several localities, were collected in small large-mouthed bottles fitted with screw caps. The samples were taken from the upper four or five centimeters of the soil since experience had shown that very few myxobacteria were to be found below that depth. Dung that had lain on the ground for some time was found to harbor larger numbers of myxobacteria than fresher material. The soil, or dung, after being collected, was broken up into small particles. Sieving of the soil was often resorted to in order to eliminate stones, grass, and other undesirable materials.

Petri dishes were prepared by placing two or three pieces of filter paper in the bottoms, to aid in retaining the moisture, and enough of the sample added to approximately half fill the plate. Twenty-five to forty pieces of fresh rabbit dung were then placed on top of the soil, or dung, and water added up to 75 to 90 per cent saturation. Various incubation temperatures were tried, but the most satisfactory was found to be room temperature, 20° - 25° C. When held there the development of fruiting bodies on the dung was more normal, though possibly a little slower, than at a somewhat higher temperature.

During the first three to four days, molds developed, in some cases in such large numbers as to make the plates of no further use. Usually the molds reached maximum growth within five days, after which fruiting bodies of some species of myxobacteria began to develop. Plates not completely overgrown by molds were examined daily under a low-power binocular microscope, and when myxobacterial fruiting bodies '

were found they were transferred to plates of dung decoction agar that had previously been prepared.

This medium was made by adding to 100 grams of fresh rabbit dung one liter of distilled water. The mixture was heated to boiling, then allowed to infuse for 24 hours, filtered through several layers of cheese cloth, the filtrate made up to volume, and 1.5 per cent Bacto agar added. Sterilization was effected by autoclaving at 15 pounds pressure for 30 minutes. When cooled to about 60° C. plates were poured.

The primary transfers, of which 127 were made from the various soils and dungs, were, of course, not pure cultures. Often molds were carried along on the needle, and these frequently outgrew the myxobacteria. Associated bacteria of various types were also present and usually developed first. These, too, occasionally grew rapidly enough to completely inhibit the growth of the more slowly growing slime bacteria. However, fruiting bodies were sometimes able to develop, and were first seen on the tops of colonies of the associated types. Attempts to transfer parts of these fruiting bodies to new media usually resulted in transferring both the myxobacteria and the associate. The appearance of some of these primary transfers after seven to ten days incubation is shown in Figures 1, 2, and 4. The fruiting bodies are seen to be developing on the surface of colonies of associated types. By allowing a longer period of incubation pure cultures could be obtained from the thin, nearly transparent vegetative slime colony that developed beyond the margins of the colonies of associated bacteria. Two weeks incubation were often required for the development of these vegetative colonies, and the fruiting bodies that eventually formed on them were usually smaller and less perfect than those produced directly on the host colony. This fact may be noted in Figure 1. These observations led to the development of a somewhat improved method for isolation.

After molds had been eliminated, fruiting bodies were transferred to a medium made by growing large numbers of true bacteria (*Escherichia coli*, *Bacillus subtilis*, *Serratia marcescens*, etc.) in flasks of nutrient broth. After a good growth had been obtained the suspensions were shaken well, in order to distribute the cells evenly throughout the suspension, and 1.5 per cent agar added. The flasks were then autoclaved at 15 pounds pressure for 30 minutes. Transfers were then made to plates of this medium. The growth of the myxobacteria was greatly enhanced, and the colonies of vegetative cells developed much more rapidly than did those of associated types. A later modification of this method consisted of growing large numbers of true bacteria on agar, scraping the growth from the agar and washing free of any adhering nutrient materials. An angle-head centrifuge was used for the purpose. After three washings in distilled water the cells were concentrated, the supernatant liquid poured off, and the paste dried *in vacuo*. The dried cells were then suspended in 1.5 per cent plain agar (no other nutrient

materials were added), and the medium sterilized as usual. This medium produced vigorous growth of the myxobacteria, while the associated species failed, in nearly every case, to develop at all. This procedure shortened the isolation period to a few days, rather than two or three weeks.

When pure cultures had finally been obtained they were transferred to dung plates. These were prepared by placing two or three pieces of rabbit dung in each petri dish and sterilizing for 1 hour at 15 pounds pressure in the autoclave. A 1.5 per cent solution of Bacto agar in distilled water was added to each plate to a depth equal to about one-half the diameter of the pellets of dung. The dung was then moved to the center of the plates with a sterile needle or forceps before the agar solidified. Care was taken not to cover the dung, but to keep the top side of each piece free from agar. Much better growth could be had directly on the dung than on agar-incrusted dung. Transfers were made to the dung, the agar acting to hold it in place and to preserve the moisture content. Growth of all species was found to be good on this medium, and the fruiting bodies produced were large and appeared more nearly normal than on most other media that had been tried. This medium has been used successfully for carrying stock cultures over periods of many months. Transfers are necessary about once every six to eight weeks, depending upon humidity and temperature.

SPECIES ISOLATED

Undoubtedly conditions vary sufficiently from one country to another to affect, to a high degree, the flora of the soils. It has been the experience of the present author that the majority of types to be found in the soils in the environs of Ames, Iowa, belong to the genus *Myxococcus* and that other types, while present, are not abundant. Private communications with two other persons interested in the group tend to indicate that this is also true in other parts of the United States. Many of the species used for this work were originally found on dung.

Of the 127 primary transfers made, only a small percentage were finally isolated in pure condition. This was chiefly due to the fact that the associated organisms (both bacteria and molds) were able to outgrow the myxobacteria after transfer from the natural substrate. It is of interest to note the variety of types that were observed, with some indication of the natural substrate. A condensed list is given here, with brief descriptions of the species that were isolated in pure culture. These are indicated by the culture numbers following the names.

SPECIES CULTURED

Myxococcus fulvus (Cohn *emend.* Schroeter) Jahn 1, 44, 76, 106. Produces bright pink fruiting bodies, spherical to subspherical, often constricted at the base, and supported on a mound, or foot, of slime; the surface smooth with no outer wall or limiting membrane; up to 350 μ in

diameter. Spores spherical, average 1.3 μ diameter.

From wet sand, pasture soil, cow, sheep, and goat dung.

Myxococcus fulvus var. *albus* Jahn 108. Similar to *M. fulvus* but color much lighter, often giving rise to very pale pink or nearly white fruiting bodies.

Myxococcus virescens Thaxter 57, 61, 91, 100. Fruiting bodies somewhat smaller and less regularly spherical than those of *M. fulvus*. Color yellow to greenish yellow. Spores 2.0 μ diameter.

From wet sand, pasture soil, goat dung.

Myxococcus xanthus[2] Beebe 115, 127. Similar to *M. virescens* in size and shape of fruiting bodies and spores. Color bright orange.

From pasture soil and cow dung.

Polyangium fuscum (Schroeter) Thaxter 77. Fruiting bodies composed of masses of oval to spherical cysts, each surrounded by a tough, reddish-brown wall; up to 90 x 125 μ. Mass of cysts held together by a colorless, transparent slime envelope. Resting cells, or spores, rod-shaped; about 0.8 x 3.0 μ.

From sand, sandy loam, pasture soil, cow, sheep, and rabbit dung.

Podangium erectum (Schroeter) Jahn 82, 104. Reddish-brown cysts, oval or club-shaped, rising from a sort of hypothallus. Cysts single, each with a thick outer wall or membrane; 40 - 50 μ in diameter, up to 100 μ in height. Often 100 or more on one hypothallus. Spores shortened rods, 0.7 x 3.0 μ.

From cow dung and soil from woods and pasture.

Chondrococcus Blasticus, sp. n. 90, 111.

This organism was at first considered a large variety of *M. fulvus* due to the marked similarity of the primary fruiting body of this species, both in color and formation, and those of *M. fulvus*.

ETYMOLOGY: Greek adj. = budding, additional growth.

DIAGNOSIS

FRUITING BODY. Primary: Spherical to subspherical, usually sessile but occasionally with a short stalk or foot, pale to bright pink; 300 to 600 μ in diameter. No outer wall or limiting membrane evident. Develops on sterilized rabbit dung in from 3 to 5 days at room temperature. Secondary: Arising as a budlike growth from the primary fruiting body. Develops into irregularly shaped, finger, coral, or budlike protuberances. Seldom branched, occasionally stalked but usually sessile on primary fruiting body. Deep pink to salmon pink in color. Quite variable in size and shape: 50 - 150 x 175 - 425 μ. No outer wall or limiting membrane evident.

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[2]Diagnosis of *M. xanthus* included in paper submitted to Journal of Bacteriology; in press at the time of this writing.

SPORES. Spherical, thick-walled, highly refractile; 1.2-1.4 μ in diameter. Held together in the fruiting body by the mass of slime.

VEGETATIVE COLONY. Thin, colorless, transparent at margin; surface broken by many small ridges, or veins. Center smoother, slightly thicker, often showing pale pink pigmentation.

VEGETATIVE CELLS. Long, slender, flexible rods, straight or curved to bent, ends rounded to tapered, gram negative. Often show one or two deep-staining bodies within, at or near center, while ends of cell stain lightly. 0.5 - 0.6 x 3.0 - 5.0 μ. Usually found in groups of from 2 to 12, lying parallel, the group moving as a unit. Motile by a crawling or creeping motion; no flagella.

HABITAT. Observed once on goat dung, once in soil, Ames, Iowa.

SPECIES IDENTIFIED BUT NOT CULTURED

Chondromyces crocatus Berkeley and Curtis from cow dung.

Chondrococcus coralloides (Thaxter) Jahn from pasture soil and cow dung.

Chondrococcus sp. (yellow) from sheep dung.

Myxococcus sp. (deep red-orange) from sheep dung.

Myxococcus fulvus and *M. virescens* were found to be by far the most numerous species, the fruiting bodies appearing in large numbers on plates of nearly every sample brought into the laboratory. *Polyangium fuscum* was noted on a wide variety of soils and dungs, but always in small numbers, as was the case with *M. xanthus* and *Podangium erectum*. *Chondromyces crocatus* appeared in moderately large numbers on only one sample of cow dung. Attempts to purify this species failed completely, as was the case with *Chondrococcus coralloides* and undetermined species of *Chondrococcus* and *Myxococcus*. *Myxococcus fulvus* var. *albus* was found only once in sandy soil.

Several authors have reported various species of myxobacteria from such materials as bark of trees, dead leaves, straw, etc. These materials were all sampled during the course of the work but no slime bacteria were isolated from any of them.

SUMMARY

An improved method for the isolation of myxobacteria from soils and dungs is described.

A new species, *Chondrococcus blasticus*, is diagnosed.

The distribution of species of the *Myxobacteriales* in Iowa soils in the vicinity of Ames is discussed and shown to be fairly general as regards the families *Myxococcaceae* and *Polyangiaceae*. By far the most common species were those belonging to the genus *Myxococcus*, particularly *M. fulvus* and *M. virescens*. *M. xanthus* also appeared on a wide variety of soils and dungs, but in smaller numbers. This was also the case with *Polyangium fuscum* and *Podangium erectum*. No species of

the families *Sorangiaceae* or *Archangiaceae* were observed, though it is not suggested that they are entirely absent from the soils of this region.

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PLATE I

1. Fruiting bodies of *Myxococcus fulvus* produced on a host colony. The vegetative myxobacterial colony may be seen to extend beyond the margin of the host colony, and to have formed smaller fruiting bodies directly on the agar. 20 ×.

2. *M. fulvus* fruiting bodies on a different type of host colony. Note the tendency of the fruiting bodies to form on the eubacterial host colony rather than on the agar. 10 ×.

3. Pure culture of *M. fulvus* growing on rabbit dung plate. The fruiting bodies shown are on the surface of the agar around the imbedded piece of dung. 20 ×.

4. *Myxococcus virescens* growing on and around a colony of true bacteria. 10 ×.

PLATE I
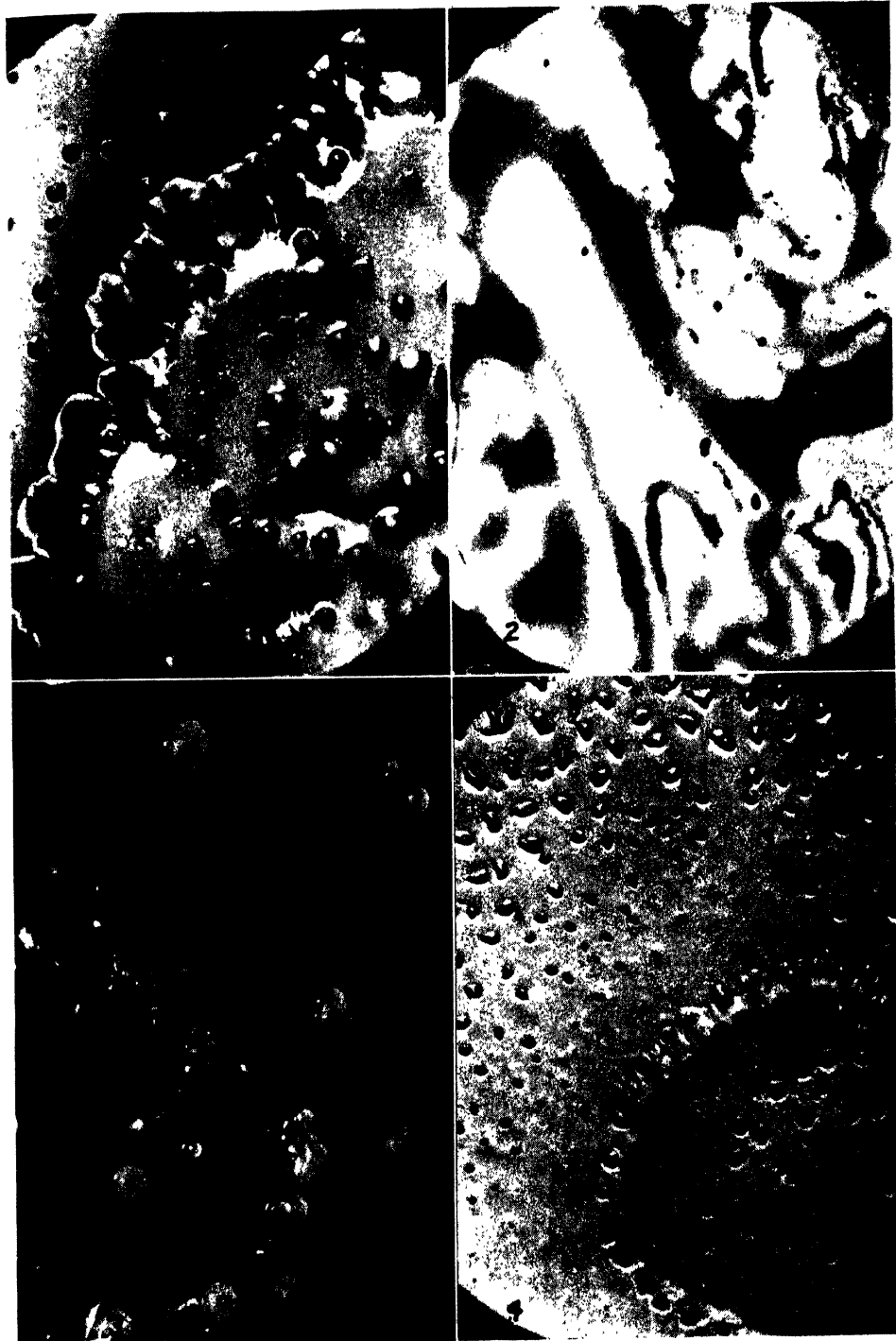
PLATE II

5. Immature fruiting body of *Chondrococcus blasticus* sp. n. The secondary fruiting bodies are shown developing from the large primary. On sterilized rabbit dung. 50 ×.

6. Mature fruiting body of *C. blasticus*. The large primary fruiting body has been completely utilized in the formation of the secondaries. 50 ×.

7. Several stages in the formation of cysts of *Polyangium fuscum*. The fruiting body first forms as a mass of colorless slime containing the long, flexible, rod-shaped bacteria. The cells then begin to group themselves at various points to form cysts, which become differentiated within the slimy mass. An early stage is shown near the lower right corner of the illustration; the cyst wall has not yet formed. Near the opposite corner are cysts in a more advanced stage, the cyst wall forming, but as yet unpigmented. Cysts near the center are nearly mature and show large pigmented areas in the walls. 125 ×.

8. Mature cysts of *P. fuscum*. These are much larger than those shown in Fig. 7, and the pigment is evenly distributed throughout the walls. The slime envelope which holds the mass of cysts together is shown in both photographs. About 150 ×.

PLATE II

5

6

7

8

PLATE III

9. Clumps of fruiting bodies of *Podangium erectum* growing on agar. Oval or club-shaped cysts may be noted. 50 ×.

10. Cysts of *P. erectum* cleared in xylol and mounted in immersion of oil. The moderately thick cyst walls may be seen, as well as the shortened, rod-shaped cells within. 430 ×.

PLATE III
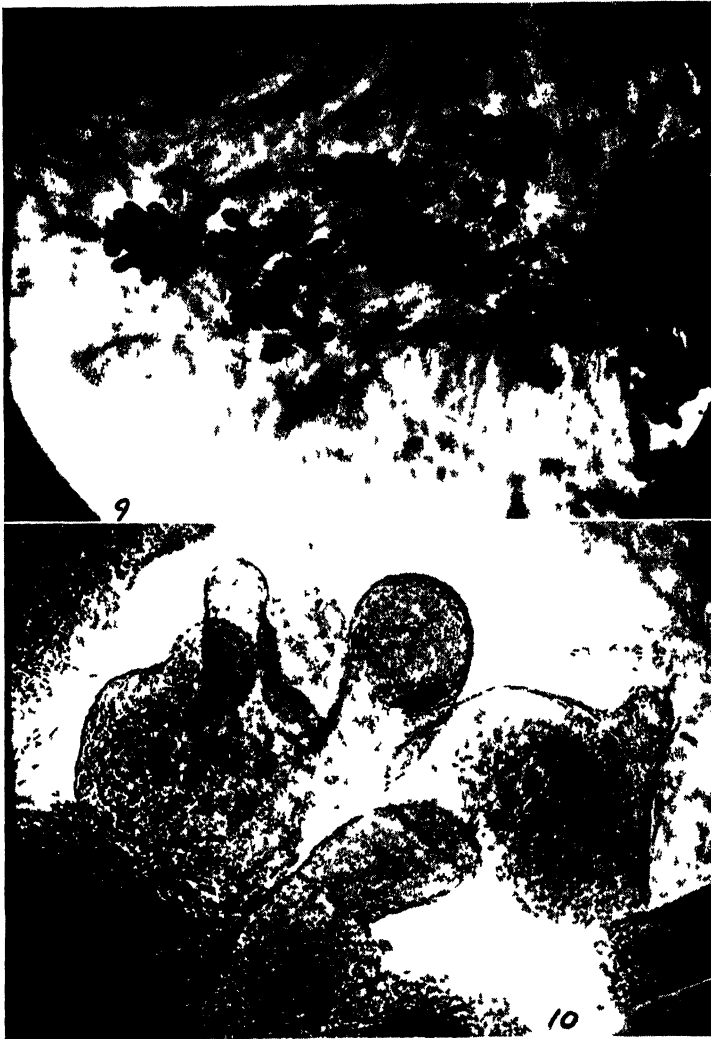
STUDIES ON THE MYXOBACTERIA

2. The Role of Myxobacteria as Bacterial Parasites

Few of the investigators who have concerned themselves with the myxobacteria have studied the nutritional requirements of the members of this group. The earlier workers observed that most species developed well on dung of one kind or another. Quehl (1906) recorded slow growth of some species which he cultivated on malt extract gelatine, the gelatine becoming liquefied. Good growth occurred on potato nutrient agar, but sterilized potato was not a satisfactory substrate. Kofler (1913) described good growth on a sucrose-peptone agar containing small amounts of magnesium sulphate and potassium phosphate. He regarded peptone as necessary for normal development.

Pinoy (1913) made first mention of a possible symbiotic or parasitic relationship of certain myxobacteria with species of true bacteria. He noted that *Chondromyces crocatus* failed to develop on any medium unless some species of *Micrococcus* was also present. He concluded the presence of the latter was necessary for the growth of the former.

In a report on the utilization of various carbon compounds by certain species of the *Myxobacteriales,* Beebe (1940) showed the use of both starch and cellulose, the former being broken down by a diffusible extracellular enzyme. He also noted a dependence of the myxobacteria on other bacterial forms: killed suspensions of true bacteria were found to supply all of the nutrient requirements of the slime bacteria. This was suggested as being parasitism rather than symbiosis, and was borne out by the findings of Snieszko, McAllister and Hitchner (1941) who pointed out a lytic action of certain species of myxobacteria on living colonies of gram-negative true bacteria.

The purpose of the present work was to determine, if possible, something of the nature of the nutritional demands, with special emphasis on the relationship with true bacteria, of several species of myxobacteria from soils and dungs of Iowa.

ISOLATION PROCEDURES

The methods used in the isolation of the various species have been previously outlined. In brief they were as follows:

Soil and dung samples, collected in screw-cap jars, were pulverized and sieved, and distributed in petri dishes to a depth of about one-quarter inch. Twenty-five to forty pieces of rabbit dung were placed on the sample and water added to about 75 to 90 per cent saturation. The plates were incubated at room temperature (22° - 25° C.) for ten to fourteen days. Molds developed rapidly the first three or four days of incubation, but after their maximum period of growth had passed,

fruiting bodies of the myxobacteria began to form on the dung. The plates were examined daily under a low-power binocular microscope. When fruiting bodies were located they were transferred to plates of dung decoction agar. If the fruiting body germinated, and was not over-grown with associated molds and bacteria, an additional transfer to a bacterial cell suspension agar usually completed the isolation procedure. Stock cultures were held on plates of sterilized rabbit dung partially imbedded in plain 1.5 per cent agar.

ASSOCIATED BACTERIA

Forty-six isolations were made of the organisms found associated with the myxobacteria in order to determine whether any particular type played an important part in the growth and development of the slime bacteria. Of the total number only five were spore-forming rods; two were cocci. The remainder were gram-negative, non-spore-forming rods varying in shape from nearly spherical cells less than a micron long up to rods nearly 4 μ long. In only a very few cases did the pigmentation vary decidedly from white or light yellow; one culture produced pale orange pigment, a few developed a bright yellow color after several days, and two were pale pink or pinkish orange. Except in the case of the cocci, which belonged to the genus *Staphylococcus*, and in the case of one spore-former, the cells were all single, never forming chains or any other definite type of group. On the whole they represent common soil forms; they have not as yet been further identified.

Several of these associated forms were used during the succeeding work. Each has been identified by a number indicating its source, such as Associate No. 100/3: the third associate cultured from primary isolation No. 100.

EXPERIMENTAL PROCEDURES AND RESULTS

Early observations showed what appeared to be a close relationship between the myxobacteria and certain true bacteria. Growth of such organisms as *Chondromyces crocatus* in mixed culture, especially in the presence of various bacteria and molds, and the ability of this organism to develop only poorly, if at all, under other conditions, checked closely with the work of Pinoy (1913) on the same species. *Myxococcus vires-cens* also showed a marked tendency to degenerate when in pure cul-ture, but to revive and produce more or less normal fruiting bodies when again transferred to unsterilized rabbit dung. *Polyangium fuscum* presented a problem in isolation in that it appeared to thrive in what seemed to be an extreme case of association: a very small, motile, gram-negative, non-spore-forming rod was noted to live in the slimy outer envelope surrounding the ripened cysts, as well as in the slime of the vegetative colony. Both organisms seemed to benefit by the association and it was only after many months that a separation was effected. The associate failed to develop on any other medium. *P. fuscum* was found to

grow well without it when inoculated on sterilized rabbit dung, or a bacterial suspension in agar. The development of large, perfectly formed fruiting bodies by most of the species on the tops of colonies of associated true bacteria, while those developing beyond the margins of the host colonies were smaller and less perfect, also indicated the importance of the relationship.

However, it was felt that some other factor might have an important role in the nutrition of the slime bacteria; the frequent reference of the earlier workers to growth on dung of all kinds might mean the utilization of fecal types of bacteria by the myxobacteria, the presence of some sort of growth factor in dung (aside from bacteria) particularly favorable for the growth of myxobacteria, or a combination of both.

After several preliminary trials a series of experiments was run in an effort to determine whether the dung itself was necessary to the growth and development of certain species, or whether the factor influencing the myxobacteria was to be found in the bacteria said to compose a large part of dung. Four media were used: (1) nutrient agar containing the usual 3 grams of beef extract, 5 grams of peptone, and 15 grams of agar per liter; (2) dung decoction agar, made as previously described; (3) bacterial suspension-nutrient agar which was prepared by inoculating 1 liter of nutrient broth with an actively growing broth culture of associate No. 91/3, a large, non-spore-forming rod. This was incubated at 37.5° C. for 48 hours, during which time it was shaken frequently for purposes of aeration. 1.5 per cent agar was then added and the suspension autoclaved for 30 minutes at 15 pounds pressure. This medium contained both bacterial cells and their metabolic products. (4). Bacterial suspension agar was prepared by growing the same organism, No. 91/3, on large slants (1 x 8 inch-tubes) of nutrient agar. After three days incubation at 37.5° C. the heavy growth of 20 slants was removed, suspended in distilled water used for washing the slants clean, and concentrated by centrifugation. The cells were re-washed and centrifuged twice in order to remove any adhering nutrient materials or metabolic products. The washed cells were suspended in 250 cc. of distilled water and plate counts made to compare the concentration of cells with that of the bacterial suspension-nutrient agar medium, plate counts having been previously made on the latter. The cell counts on the washed suspension averaged about twice those made on the nutrient broth suspension. Accordingly, the washed cell suspension was diluted to twice its volume, 500 cc., and 7.5 grams of agar added. Sterilization was in the autoclave at 15 pounds pressure for 30 minutes.

All of these media were used for plate cultures. Inoculations were made by transferring matured fruiting bodies from the stock cultures, rabbit dung plates, by means of a fairly short needle. In order to facilitate the transfer a low-power binocular microscope was used; this made it possible to pick up about the same amount of material on the needle

for each transfer, and eliminated, to a large degree, carrying over bits of dung or agar from the stock cultures. The fruiting bodies were implanted on the centers of the plates. Incubation for this first work was at 30° C. for periods of from six to ten days. As a rule 12 to 72 hours were required for the germination of the spores before any growth could be noted; this period depended chiefly on the species, rather than temperature or medium.

The following species were used in this experiment:
    Myxococcus fulvus 44, 76.
    M. virescens 57, 61.
    Polyangium fuscum 77.
    Podangium erectum 82, 104.
    Chondrococcus blasticus 90, 111.

The results after seven days are shown in Tables I - IV. Growth was compared, by measuring the diameters of the colonies in millimeters, each day during the incubation period; formation of fruiting bodies is indicated by figures in boldface.

TABLE I

DIAMETERS OF COLONIES IN MILLIMETERS ON BACTERIAL SUSPENSION AGAR
SEVEN DAYS—30.0° C.

NAME AND NUMBER	AGE IN DAYS					
	2	3	4	5	6	7
M. fulvus 44	12.5	20.5	28.0	**35.0**	**40.0**	**46.0**
M. virescens 57	6.4	16.0	25.0	**30.0**	**38.0**	**47.0**
M. virescens 61	6.4	13.5	20.0	**25.0**	**28.0**	**33.0**
M. fulvus 76	14.0	22.5	32.0	**36.0**	**42.5**	**46.0**
P. fuscum 77	8.5	8.5	10.0	....	....	......
P. erectum 82	3.5	5.0	10.0	**17.0**	**23.0**	**30.0**
C. blasticus 90	7.2	16.0	23.0	**30.0**	**33.0**	**38.0**
P. erectum 104	3.2	9.5	18.0	**30.0**	**38.0**	**45.0**
C. blasticus 111	12.0	13.0	25.0	**30.0**	**34.0**	**40.0**

TABLE II

DIAMETERS OF COLONIES IN MILLIMETERS ON NUTRIENT AGAR
SEVEN DAYS—30.0° C.

NAME AND NUMBER	AGE IN DAYS					
	2	3	4	5	6	7
M. fulvus 44	6.5	10.0	15.0	17.5	20.0	22.5
M. virescens 57	5.6	12.0	18.0	**23.0**	**27.0**	**33.0**
M. virescens 61	6.1	19.0	15.0	18.0	21.0	**25.0**
M. fulvus 76	6.4	9.0	11.5	12.5	13.0	14.0
P. fuscum 77	9.5	10.0	10.0	....	....	......
P. erectum 82	1.5	1.6	5.0	**11.0**	**16.5**	**24.0**
C. blasticus 90	3.6	6.0	8.5	10.0	**11.5**	**14.0**
P. erectum 104	....	....	....	....	....	......
C. blasticus 111	4.5	8.0	13.0	14.5	16.0	17.5

TABLE III

DIAMETERS OF COLONIES IN MILLIMETERS ON BACTERIAL SUSPENSION-NUTRIENT AGAR
SEVEN DAYS—30.0° C.

NAME AND NUMBER	AGE IN DAYS					
	2	3	4	5	6	7
M. fulvus 44	10.0	13.0	20.0	....	....	......
M. virescens 57	9.0	14.0	21.0	26.0	28.5	33.0
M. virescens 61	9.6	15.0	18.0	25.0	30.0	36.0
M. fulvus 76	3.5	11.5	15.0	16.0	17.5	18.0
P. fuscum 77	....	....	....	....	....	......
P. erectum 82	3.2	3.5	3.5	12.0	19.0	25.0
C. blasticus 90	6.0	13.0	20.0	24.0	26.0	28.0
P. erectum 104	....	....	....	....	....	......
C. blasticus 111	8.5	15.0	21.0	25.0	29.0	32.0

TABLE IV

DIAMETERS OF COLONIES IN MILLIMETERS ON DUNG DECOCTION AGAR
SEVEN DAYS—30.0° C.

NAME AND NUMBER	AGE IN DAYS					
	2	3	4	5	6	7
M. fulvus 44	1.6	2.0	3.0	6.0	7.0	8.0
M. virescens 57	3.6	4.5	8.0	11.0	14.0	21.0
M. virescens 61	5.6	8.0	13.0	17.0	20.0	27.0
M. fulvus 76	5.5	8.0	11.0	15.0	18.0	23.0
P. fuscum 77	6.0	6.2	7.0	....	21.0	31.0
P. erectum 82	3.6	4.5	5.2	7.0	7.5	7.5
C. blasticus 90	....	....	....	....	....	......
P. erectum 104	3.5	3.7	7.0	15.0	20.0	34.0
C. blasticus 111	2.4	2.4	2.4	....	....	......

In order to compare more easily the growth of any one organism
on the four media, the figures for several of the cultures given above
have been plotted as growth curves. These are given in Figures 11 to 16.
With two strains of *Myxococcus fulvus* better growth was obtained on
the suspension of killed cells alone. In culture No. 44 the second best
growth was on the nutrient agar-bacterial suspension combination, fol-
lowed by plain nutrient agar and lastly by dung decoction agar. With
culture No. 76 the three last named media closely approximated each
other, all being much less favorable than the bacterial suspension alone.
Myxococcus virescens 57 and 61 presented less striking pictures, al-
though in one case there is a significant difference between the colony
size on the suspension of killed cells and that on the nutrient agar, and
bacterial suspension-nutrient agar, both of which appeared to produce
identical results. Dung decoction agar ranked third in the case of No.
61, fourth in the case of No. 57. *Polyangium fuscum* 77 failed to grow
in three instances, developing only moderately well on dung decoction
agar. *Podangium erectum* 82 and 104 showed particularly significant
differences in rates of growth on bacterial suspension agar and dung

MYXOCOCCUS VIRESCENS 57

o — Cell suspension agar
● — Cell suspension-nutrient agar
▽ — Dung decoction agar
▶ — Nutrient agar

DIAMETERS OF COLONIES IN m.m.

AGE IN DAYS

Fig. 12

MYXOCOCCUS FULVUS 44

o — Cell suspension agar
● — Cell suspension-nutrient agar
▽ — Dung decoction agar
▶ — Nutrient agar

DIAMETERS OF COLONIES IN m.m.

AGE IN DAYS

Fig. 11

CHONDROCOCCUS BLASTICUS 90

o —— Cell suspension agar
● —— Cell suspension-nutrient agar
▽ —— Dung decoction agar
▼ —— Nutrient agar

DIAMETERS OF COLONIES IN m.m.

AGE IN DAYS

Fig. 14

PODANGIUM ERECTUM 82

o —— Cell suspension agar
● —— Cell suspension-nutrient agar
▽ —— Dung decoction agar
▼ —— Nutrient agar

DIAMETERS OF COLONIES IN m.m.

AGE IN DAYS

Fig. 13

CHONDROCOCCUS BLASTICUS 111
o — Cell suspension agar
● — Cell suspension nutrient agar
▽ — Dung decoction agar
▼ — Nutrient agar

DIAMETERS OF COLONIES IN mm

AGE IN DAYS

FIG. 16

MYXOCOCCUS FULVUS 106
o — Cell suspension agar
● — Cell suspension nutrient agar
▽ — Dung decoction agar
▼ — Nutrient agar

DIAMETERS OF COLONIES IN mm

AGE IN DAYS

FIG 15

decoction agar. Culture number 82 gave identical results on the two nutrient agars, while 104 failed to develop on either. *Chondrococcus blasticus* 90 and 111 both grew rapidly, in a nearly straight line curve, on the suspension of killed cells alone, with no apparent decrease in rates of growth during the entire incubation period. On the nutrient agar-cell suspension medium there is seen to be a definite falling off in rate after 4 days, while the same is true on nutrient agar. The entire latter curve is much lower than either of the preceding. This organism failed to grow on dung decoction agar.

The production of fruiting bodies may also be considered indicative of the value of a particular medium. Seven of the nine strains produced fruiting bodies on cell suspension agar at the end of the period of incubation, while one, *M. fulvus* 76, had fruited by the third day. Five produced fruiting bodies by the fifth day. At the end of the seven-day run only three cultures showed fruiting bodies on nutrient agar, and two on each nutrient-cell suspension and dung decoction agars.

A second series of experiments was run to check the first. This time associates number 82/1 and 100/3, a short, gram-negative rod and a *Staphylococcus* respectively, were used in the suspensions. It was thought that the presence of the metabolic products might have some inhibitory effects on the growth of the myxobacteria so that the cells to be suspended were grown on large slants, the growth removed as previously described, and re-suspended in distilled water to which was added 7.5 grams of plain agar in one case, 11.5 grams of prepared Difco Bacto Nutrient agar in the other, to each 500 cc. of suspension. The results, given in condensed form in Table V, after seven days incubation at 30° C., were essentially the same as in the previous trials.

TABLE V

DIAMETERS OF COLONIES IN MILLIMETERS ON FOUR CELL SUSPENSION MEDIA
SEVEN DAYS—30.0° C.

NAME AND NUMBER	ASSOCIATES			
	82/1		100/3	
	PLAIN	NUTR.	PLAIN	NUTR.
M. fulvus 44	17	....	15	....
M. virescens 57	27	19	19	25
M. virescens 61	24	27	26	28
M. fulvus 76	22	6	17	12
P. fuscum 77	31	19	23	....
P. erectum 82	42	28	37	24
C. blasticus 90	25	10	22	10
P. erectum 104	37	....	15	9
C. blasticus 111	26	13	21.	14

Except for *M. virescens* 57 and 61, best growth invariably occurred on the suspension of short rods (No. 82/1), and the next most favorable medium in every case was the suspension of *Staphylococcus* No. 100/3.

A comparison of the two nutrient agars will show little difference with the type of suspended cells. Fruiting body production was higher on the two plain cell suspensions, although there was less difference to be seen here than in the previous trials.

Within 24 hours after the inoculations on the suspensions of cells, a clarified area, or window, could, in most cases, be noted around the point of inoculation. As the colonies grew, this area was seen to increase in size. The cells in the suspensions appeared to be lysed by extracellular enzymes secreted by the myxobacteria. Microscopic examination of agar from within this area showed very few cells remaining in the medium, while the agar outside the growth area revealed the presence of numerous cells intact. In a few cases the lysed area extended several millimeters beyond the margin of the myxobacterial colony, but for the most part the diameters of the two coincided.

Quantitative determinations were then made in an effort to show the effect of varying the concentrations of the cell suspensions. Trial runs, using associate culture No. 100/3 again, indicated a direct relationship between the number of cells in the suspension and the rates of growth of the myxobacteria. In these first experiments the cells for the suspensions were grown on large slants and suspended in distilled water to give concentrations of 80,000,000; 40,000,000; and 20,000,000 cells per cubic centimeter. These were determined by plate counts. No other nutrient materials were added to the suspension in agar. Fruiting body production on the three media was, in general, about the same throughout. This was thought to be due to too little difference between the highest and lowest cell concentrations.

A series of more closely controlled experiments followed. It was felt that better control could be exercised with definite weights of cells rather than approximate numbers such as result from plate counts. Large numbers of cells were grown in flats—six-ounce Blake bottles to which had been added 20.0 cubic centimeters of nutrient agar; these were plugged with cotton, sterilized as usual, and allowed to cool in a horizontal position, giving a large surface of nutrient agar upon which to grow the cells. Inoculations were made by pipette from actively growing 24-hour broth cultures, and the flats incubated at 37.5° C. for two days. The growth was then scraped off, suspended in distilled water, centrifuged, and then re-washed and centrifuged two additional times. The resulting cell paste was dried in vacuo and stored in a desiccator over calcium chloride.

The following cultures were so treated and used for cell suspensions:

  No. 100/4—Large spore-forming rods, colorless.
  No. 89/2—Small spore-forming rods, colorless.
  No. 108B/3—Sarcina, yellow.
  No. AB—(a contaminant)—Serratia, red.

The purpose of this variety was to determine the effects, if any, of spore-

formers and chromogens on the growth of myxobacteria. The cells were ground lightly in an agate mortar to reduce the dried paste to a powder, and added to 1.5 per cent plain agar in the following concentrations:

        00.0 milligrams per 100.0 cc. agar (control).

        25.0 milligrams per 100.0 cc. agar

        50.0 milligrams per 100.0 cc. agar

     100.0 milligrams per 100.0 cc. agar

The first trial was with suspension 100/4, and the results of this experiment over a seven-day period are shown in Table VI. *Myxococcus fulvus* 76 and 108, *Podangium erectum* 82, *Chondrococcus blasticus* 111, and *Myxococcus xanthus* 127 were the species used for this experiment.

TABLE VI

DIAMETERS OF COLONIES IN MILLIMETERS ON VARIOUS CONCENTRATIONS OF A
SPORE-FORMING BACTERIUM SEVEN DAYS—ROOM TEMPERATURE

MGM. OF CELLS/ 100 cc.	CULT. No.	AGE IN DAYS			
		1	3	5	7
00.0	76	1.5	3.7	6.0	7.0
	82	1.0	1.5	2.0	7.0
	108	2.0	2.3	2.5	2.5
	111	1.5	2.0	2.0	2.5
	127	1.5	3.5	5.0	7.0
25.0	76	3.2	8.0	12.0	16.0
	82	2.0	3.0	5.0	6.0
	108	2.0	2.2	5.0	8.0
	111	2.0	7.2	12.0	18.0
	127	......	5.7	10.5	15.5
50.0	76	3.0	9.0	14.0	19.0
	82	1.5	1.5	2.0	12.0
	108	1.5	2.5	6.5	11.0
	111	2.5	8.5	15.0	21.0
	127	2.0	7.0	13.0	19.0
100.0	76	2.5	9.0	14.0	19.0
	82	2.0	3.0	5.0	11.5
	108	2.0	3.5	8.0	14.0
	111	2.0	9.5	16.5	22.0
	127	2.0	9.5	18.0	23.0

Examination of the data reveals a gradual but definite increase in the diameters of the colonies, in nearly every case, proportional to the increase in the cell content of the medium. There is also a corresponding tendency to produce fruiting bodies in the higher cell concentrations, while none was formed in the lower ones. It also might be noted here that with *M. fulvus* 76, *P. erectum* 82, and *M. xanthus* 127, growth actually occurred on the plain 1.5 per cent agar which acted as the control. It was thought that the distilled water used in the solutions might possibly contain sufficient minerals to support growth. Additional trials were made using glass-distilled water, but growth on those media closely approximated that shown in the above table.

Table VII gives, in condensed form, the results of growth on the three other cell suspensions previously mentioned. The incubation period was also seven days, and the plates held at room temperature. *Myxococcus fulvus* 44 was substituted for 76; all other cultures were the same.

TABLE VII

SUMMARY OF GROWTH ON VARIOUS CONCENTRATIONS OF THREE CELL SUSPENSIONS; DIAMETERS OF COLONIES IN MILLIMETERS; SEVEN DAYS—ROOM TEMPERATURE

CELL SUSP. No.	CULT. No.	MGM. DRIED CELLS PER 100 CC.			
		00.0	25.0	50.0	100.0
89/2	44	5.0	16.0	19.0	21.0
	82	2.0	2.5	....	28.0
	108	1.5	5.0	7.0	13.0
	111	7.0	20.0	23.0	23.0
	127	1.5	19.0	25.0	16.0
108B/3	44	....	17.0	17.0	25.0
	82	....	5.0	13.5	16.0
	108	....	5.5	11.0	16.0
	111	....	5.5	24.0	27.0
	127	....	14.0	22.0	29.0
AB	44	....	15.5	21.0	21.0
	82	....	2.0	11.5	15.0
	108	....	2.0	6.0	15.0
	111	....	12.0	26.0	27.0
	127	....	16.0	27.0	24.0

Since the various concentrations of the different suspensions were incubated at the same time, and under identical conditions, a single control was used for all. Only *Myxococcus fulvus* 44 and *Chondrococcus blasticus* 111 developed at all on the control, and then very poorly. On the three concentrations of cell suspensions the rates of growth of almost all of the organisms corresponded closely to the increase in the number of cells in the suspensions. The colonies were smallest, with one exception, on the medium containing 25 milligrams of dried cells per 100 cc. That one exception was *M. fulvus* 44 on the *Sarcina* suspension, 108B/3, and the growth rate here was the same as on the next higher concentration, i.e., 50.0 milligrams per 100 cc. Differences between growth on the 50.0 and 100.0 milligram suspensions were less marked, in many instances, some species growing less rapidly on the highest concentration. This, however, might be attributed to any one of several causes such as the size of the inoculum, which is difficult to control exactly, the manner in which it happened to be placed on the medium during the inoculation (whether it was left as a spherical fruiting body or smeared out somewhat over a larger area), etc. The fact that most of the colonies on the suspension containing 100 milligrams of cells were equal to, or larger than, those on the 50-milligram media would indicate that on the average, growth corresponded to the number of cells present.

No particular cell suspension appeared to be more favorable than any other as far as growth rates were concerned. Fruiting body production on suspension 108B/3 was somewhat better, in the highest concentration, than on the other two cell suspensions. In view of the succeeding experiment this does not appear to be significant.

The experiment was repeated using the same cell suspensions and the same species of myxobacteria. The incubation period was extended to fourteen days and the plates held at room temperature. More of the cultures produced fruiting bodies over the longer period of time, but the relationships appeared to remain the same. In every case growth on the control was poor; only *Podangium erectum* 82 produced fruiting bodies on the 00.0 concentration after fourteen days. All of the species fruited on at least one of the other three media, while *Myxococcus fulvus* 44 and *P. erectum* 82 developed fruiting bodies on every concentration of each cell suspension. There was no significant difference between the results on the three types of cells used for the suspensions, but the cell concentrations had a direct effect on rates of growth in almost every case. The difference between the two higher concentrations, 50.0 and 100.0 mgms. per 100 cc. of agar, was less marked than between the lower concentrations. It appeared that in general 50.0 milligrams of cells in 100 cc. of medium was enough to supply the needs of the myxobacteria under consideration.

In a paper read before the annual meeting of the Society of American Bacteriologists Snieszko, McAllister, and Hitchner (1941) suggested a relationship between certain species of the *Myxobacteriales* and certain gram-negative true bacteria. At about the same time that problem happened to be under consideration by the present writer. Early examination had shown most of the associated forms to be gram-negative, and the question arose as to whether or not there was a definite relationship. Since many of the soil forms are gram-negative it would be quite understandable if such were the case.

The first pair of bacteria chosen for the test, that is, to be used for the cell suspensions, was *Bacillus subtilis* and *Escherichia coli*. These were used because of both being relatively common and because either or both might possibly constitute at least a part of a natural substrate. The cells were grown in large quantities and harvested as previously described. The dried and powdered cells were added to plain agar solutions in concentrations of 100 mgm. per 150 cc. of medium. This was sterilized in the usual manner. Plates were inoculated with the following myxobacteria:

> *Myxococcus fulvus* 44
> *Polyangium fuscum* 77
> *Podangium erectum* 82
> *Chondrococcus blasticus* 111
> *Myxococcus xanthus* 127

Incubation was for ten days at room temperature. Readings, i.e., measurement of the diameters of the colonies, were made at two-day intervals. The results of the six, eight and ten-day readings are shown in Table VIII, which also includes the results of a second experiment in which another pair of bacteria was employed for cell substrates. Again one gram-positive and one gram-negative species was used, but differing from the first pair in that the cells were pigmented. They were *Sarcina* sp. and *Serratia marcescens*.

TABLE VIII

COMPARISON OF SUSPENSION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA; DIAMETERS OF COLONIES IN MILLIMETERS

AGE IN DAYS	CULT No	CELLS IN SUSPENSIONS			
		BACILLUS SUBTILIS GRAM +	E COLI GRAM —	SARCINA SP GRAM +	SERRATIA MARCESCENS GRAM —
6	44	23.0	24.0	20.5	17.5
	77	9.0	13.0	6.0	13.0
	82	20.0	26.0	30.0	33.0
	111	26.0	27.0	20.0	26.0
	127	23.0	26.0	12 0	26.0
8	44	30.0	32.0	28.0	23.0
	77	15.0	19.0	7 0	24.0
	82	40.0	45.0	46.0	50.0
	111	34 0	36 0	25.0	34.0
	127	33.0	37.0	22.0	37.0
10	44	36.0	39 0	32.0	24.0
	77	17.0	22.0	7.0	31.0
	82	57.0	64.0	66.0	60.0
	111	38 0	42.0	29.0	36.0
	127	43.0	45.0	31.0	47.0

There is shown to be a slight increase in the rates of growth of all the species on the suspension of *E. coli* over that on the suspension of *B. subtilis*. However, the difference is so small that it is doubtful whether it should be considered significant. The same is true in the case of the suspensions of pigmented bacteria. The outstanding exception here is the growth of *Polyangium fuscum* 77 on the suspension of *S. marcescens;* this is much superior to that on *Sarcina sp.* However, the growth of this particular organism has been observed, in the past, to be more or less erratic, so that this difference by itself could not be considered as of very great significance. The fact that it developed more rapidly on the non-pigmented gram-negative suspension than on the gram-positive, coupled with this large difference in rate of growth on the pigmented strains might have some bearing on the matter. If there is any difference between growth of these species of myxobacteria on gram-negative and gram-positive bacterial suspensions, the data seem to point toward the gram-negative cells as the more favorable substrate.

The effect of pigmentation, too, is rather doubtful. While many of the colonies reached a somewhat greater size on the suspensions of white cells, the actual difference between rates of growth is small. Table VII showed fruiting body production to be best on suspensions of *Sarcina* while in the present case it appears to be favored by suspensions of *Bacillus subtilis*. In general, the formation of fruiting bodies parallels, more or less directly, the concentration of cells in the medium; the kind of cells used in the suspension is of less importance.

DISCUSSION

The frequent growth on dung of the species of the *Myxobacteriales* that have been studied appears to be due, primarily, to the high bacterial content of the dung. If the water-soluble constituents of dung were necessary for growth much better development might be expected on dung decoction agar than was actually observed. This medium produced much poorer growth than even nutrient agar. The constituents utilized by the myxobacteria would obviously seem to be the water insolubles, including fecal types of bacteria. These were utilized through the agencies of bacteriolytic enzymes.

Peptone, recommended by at least one investigator as necessary for good growth, seems to have a slight, but definite, inhibitory effect when used in combination with suspension of true bacterial cells. The nature of this inhibitory action is not known, but it might affect either the myxobacterial cells themselves directly, preventing the production of enzymes, in part, or it might act on the enzyme, once it had been produced, partially or wholly destroying its activity.

In general, the kinds of associated true bacteria, acting as host cells to the myxobacteria, seem to have much less effect than the quantities in which they are present. In the absence of all other nutrient materials, with the exception of agar (which is able to support poor growth of certain species), the addition of such small amounts of dried cells as 10 milligrams per 100 cubic centimeters of medium resulted in a very definite increase in the rates of growth of all the organisms that have been studied. More than 50 milligrams of dried cells per 100 cubic centimeters of medium produced but small increases in growth rates. Under laboratory conditions this concentration of cell material seems to be optimum, rates increasing proportionally, up to this point, with the increase in concentration of cell suspension. It is possible that under natural conditions a higher concentration of host cells would be required for normal growth. The competition of the many types of soil forms would conceivably have some effect on the growth of the myxobacteria, increasing the nutrient requirements.

The gram reaction and pigmentation of the host cells may be minor factors in their utilization, though all of the myxobacterial species used in this work were able to lyse and develop upon all of the associates in

the various suspensions. It is possible that gram-negative, non-pigmented bacteria are more easily utilized by the slime bacteria, and inasmuch as such forms seem to predominate in the soil this preference could be understood. However, the myxobacteria are not especially fastidious relative to the kinds of host cells, and appear to flourish in the presence of any of the *Eubacteriales*. This relationship seems to be so definitely one of dependence that it is considered as parasitism, rather than symbiosis.

SUMMARY

The biotic relationship of the *Myxobacteriales* and *Eubacteriales* is discussed, and it is suggested that it is one of parasitism rather than strict symbiosis or association. Cells of the true bacteria are necessary for best growth and normal development of fruiting bodies by some of the myxobacteria. The host cells are destroyed by an extracellular lytic enzyme produced by the myxobacteria.

Myxobacterial growth rates and fruiting body production increase proportionally with the concentrations of killed bacterial cells in the medium, up to about 50 milligrams per 100 cubic centimeters of medium; above that point increases in rates of growth are less marked.

The evidence indicates a possible preference on the part of the myxobacteria for gram-negative, non-spore-forming, non-chromogenic bacteria, although this is not emphasized.

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PLATE IV

Fig 17. 7-day-old colony of *Myxococcus fulvus* on cell suspension agar. Lysis of the cells within area of the colony may be noted. Fruiting bodies are indicated by concentric rings of white dots. About 2 X.

Fig. 18. 5-day-old colony of *Chondrococcus blasticus* on cell suspension agar showing lysis of host cells to form a translucent "window" in the opaque medium. About 2 X.

PLATE IV
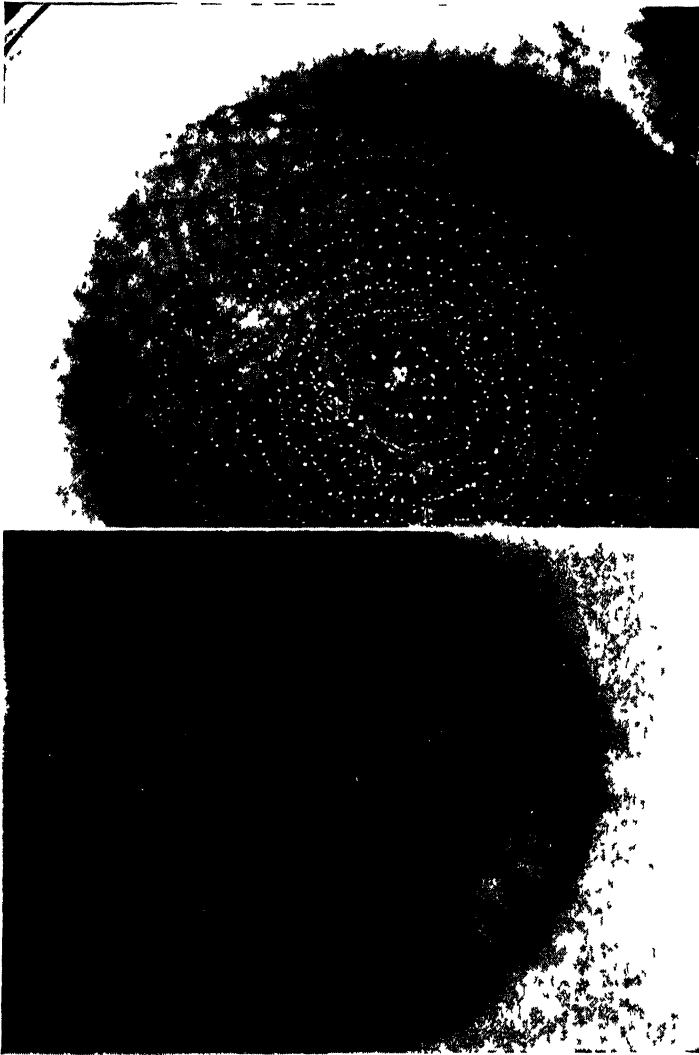
NOTE ON THE UTILIZATION OF CARBON DIOXIDE BY HETEROTROPHIC BACTERIA[1]

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Since 1935 the researches of Wood and Werkman (3, 4, 5) have shown the assimilation of carbon dioxide by heterotrophic, non-photosynthetic bacteria. In these studies species of *Propionibacterium, Escherichia,* and *Citrobacter* were used. It has been the purpose of the present investigation to determine first, the extent of carbon dioxide fixation among other heterotrophic bacteria, and secondly, the possibility of a mechanism involving carbon dioxide fixation other than the 3-carbon and 1-carbon addition made probable by Wood *et al.* (6).

In all the experiments to be reported here, cell suspensions of the various organisms were employed, with the exception of *Clostridium acetobutylicum,* which was a corn-mash culture. The substrate of *Streptococcus paracitrovorus* was 0.05 M citric acid; all other experiments contained 0.05-0.1 M glucose. Sodium bicarbonate enriched with approximately 5 per cent heavy carbon (C^{13}) was used in 0.05-0.125 M concentration. The fermented substrates were fractionated according to the methods employed in this laboratory, the products oxidized to carbon dioxide, and the C^{13} content determined by mass spectrometer analysis. In the case of *Cl. welchii,* acetic and butyric acids were separated according to the distillation method of Schicktanz *et al.* (2). Lactic acid was degraded by $KMnO_4$ oxidation to acetaldehyde and carbon dioxide. In this reaction carbon dioxide originates from the carboxyl group and acetaldehyde from the α and β carbons of lactic acid.

Reference to the table will indicate that carbon dioxide assimilation has been established with *Staphylococcus candidus, Aerobacter aerogenes, Streptococcus paracitrovorus, Clostridium welchii,* and *Clostridium acetobutylicum.* It must be remembered that naturally occurring materials contain approximately 1.09 per cent C^{13}. A value in excess of this figure indicates C^{13} carbon is present in the compound in a concentration greater than that normally occurring. Since the bicarbonate was the only source of carbon having C^{13} greater than 1.09 per cent, compounds containing a C^{13} concentration greater than this value must contain fixed carbon from the bicarbonate. Experimental variation in the determinations with

the mass spectrometer is $\pm$ 0.02. Figures indicating carbon dioxide fixation are italicized in table 1.

Lactic acid containing fixed CO_2 was formed by *Staph. candidus* and *Cl. welchii*. Substantially if not all, fixed CO_2 in this molecule lies in the carboxyl group. Acetic acid produced in the *Aerobacter* and *Cl. welchii* fermentations contained fixed carbon dioxide. Heavy carbon has been found in succinic acid in every case that it was produced, i. e., from *Staph. candidus*, *Strept. paracitrovorus* and *A. aerogenes*. All species examined have not been found to contain fixed CO_2 in the fermentation products. There was no indication of carbon dioxide fixation by *Lactobacillus plantarum*, *Lacto. acidophil-aerogenes* and *Strept. lactis*. It is interesting that certain compounds such as 2,3-butylene glycol and butyric acid, which are formed by synthesis, do not contain significant amounts of fixed carbon.

The fixed CO_2 occurring in succinic acid is believed to arise by 3 and 1-carbon addition in accordance with the Wood and Werkman reaction. With regard to the mechanism of fixation of carbon dioxide in the other products, little can be said until further information is available, particularly relative to the location of the fixed carbon atoms in the molecule. It is possible that the carbon is fixed initially by 3 and 1-carbon addition, and the final products are derived from the resulting compound. Another possibility particularly with regard to carbon dioxide fixation in the carboxyl group of lactic acid, is that of 2 and 1-carbon addition possibly involving acetylphosphate as suggested by Lipmann (1941). In this connection it is noteworthy that the lactic acid and acetic acid formed by certain bacterial species did not contain fixed carbon. This indicates a fundamental difference in the mechanism of formation of these acids among heterotrophic bacteria. The results clearly show that fixation of CO_2 is a general phenomenon among bacteria and has a wide application in their metabolism.

The location of fixed CO_2 in succinate and carboxyl group of lactic acid was shown by additional investigations with *Proteus vulgaris* to be similar to that with *Staph. candidus*.

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TABLE 1

DISTRIBUTION OF FIXED CARBON DIOXIDE CONTAINING HEAVY CARBON AMONG THE FERMENTATION PRODUCTS OF HETEROTROPHIC BACTERIA

Products		Staphylococcus candidus	Aerobacter aerogenes	Streptococcus paracitrovorus	Clostridium welchii	Clostridium acetobutylicum
Acetic acid	m. Mols. % C^{12}	54.7 1.09	160.0 1.19	137.0 1.10	32.3 1.23	34.5
Butyric acid	m. Mols. % C^{13}				16.6 1.11	33.9 $\big\}$ 1.14
Ethyl alcohol	m. Mols. % C^{13}	27.6 1.07	64.0 1.10			28.7
Butyl alcohol	m. Mols. % C^{13}				8.0 $\big\}$ 1.09	41.4 $\big\}$ 1.08
Acetone	m. Mols. % C^{13}					23.9 1.10*
Lactic acid COOH carbon	m. Mols. % C^{13}	20.0 1.34		72.4 1.10 (entire molecule)	39.3 1.68	
α and β carbon	% C^{13}	1.11			1.13	
Succinic acid	m. Mols. % C^{13}	11.1 1.25	15.4 1.42	21.9 1.31		
2, 3-Butylene glycol	m. Mols. % C^{13}		43.0 1.09			

* As 2, 4-dinitrophenylhydrazone. Yield of products expressed as m. Mols. per 100 m. Mols. substrate fermented, except Cl. acetobutylicum which is expressed as m. Mols. per liter. % $C^{13} = \dfrac{\text{moles of } C^{13}}{\text{moles of } C^{12} + \text{moles of } C^{13}} \times 100.$

A NEW GENUS AND SPECIES OF ANTHOCORIDAE (HEMIPTERA)

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In order to make a name available for the use of other workers, the descriptions given below have been extracted from our manuscript on the Anthocoridae in preparation and are presented at this time. The peculiar little species described occurs in nests of the wood-rat and in caves frequented by bats. The holotype (male) and allotype (female) are in the U. S. National Museum.

Nidicola, new genus

Small, rather oval, depressed, shiny, the head, pronotum and scutellum minutely granulose, the hemelytra very finely shagreened and with very sparse serially arranged, fine, short hairs. Head porrect, as long as broad. Eyes small. Ocelli minute, placed on a line drawn through hind margin of eyes. Antennae moderately long, segments I and II thickened, feebly pilose; III an IV very slender, clothed with long, fine hairs. Rostrum reaching between middle coxae, first visible segment reaching to a point opposite front margin of eyes; II longest, extending between anterior coxae; III slightly longer than II.

Pronotum much broader than long, the front margin slightly concave, the collar very narrow and placed entirely behind the antero-lateral angles, the disc only slightly raised, separated into two lobes by a transverse depression in front of base of scutellum, the sides very widely explanate, and somewhat reflexed, the base strongly concave. Scutellum about as long as broad, rather flat, shiny. Hemelytra rather broad, the costal areas explanate and somewhat reflexed, the clavus finely punctate, the corium also with two rows of very fine punctures. Membrane narrow, the veins obsolete, but apparently four in number. Wings undeveloped. Mesosternum small, cordate, with a fine, median longitudinal sulcus. Metasternum very small. Ostiolar canal with its rim extending outward to about middle of metapleuron, slightly curved anteriorly. Venter wide, with sparse, fine, short hairs. Legs moderate in length, with fine, short, rather indistinct hairs, the tibiae also with fine setae; tarsi three-segmented. Coxae with a row of four or five bristly hairs on antero-lateral edges, the posterior coxae approximate. Anterior femur in male fairly stout, with a row of short spinules beneath. Male genitalia asymmetrical; female with ovipositor very short.

Genotype: *Nidicola marginata*, n. sp.

Belonging to the Lyctocorinae and apparently nearest *Asthenidea* but seemingly distinct by virtue of the widely explanate pronotal and

elytral margins, the texture of the upper surface, the sparse and fine clothing, the more weakly arched rim of ostiolar canal, the short ovipositor and the armature of the front femur in the male.

Nidicola marginata, n. sp.

Yellowish testaceous, the head slightly darker, the eyes and ocelli reddish; shiny, appearing almost nude, the sparse, fine hairs showing only in certain lights. Head measured to collum as broad as long, (16:16), sharply narrowed in front of eyes, then about parallel-sided along apical third. Vertex arched. Eyes small, oblong-oval as seen from above; circular, from the side. Ocelli minute, sometimes indistinct. Antennae slender, segment I not attaining apex of head, II distinctly enlarged from the base, III and IV slender, the clothing hairs on IV longer than on others; proportions, 5:12:11:12. Rostrum long; proportions, 8:16:11.

Pronotum twice as wide as long (32:16), the anterior angles produced well in front of the very narrow collar, the disc slightly raised, the transverse impression interrupted on each side. Hemelytra widened, rather translucent, the punctures and clothing hairs indistinct in certain lights. Venter of male with a stout seta on each side of midline of third segment, the line between these setae carinate and ciliate. Sixth segment of venter of female sinuate at apex and slightly produced at mid-line over base of the short ovipositor. Left clasper of male tapering to a fine point, somewhat curved and sinuate, its length about equal to transocular width.

Length: 1.90—2.10 mm. *Width:* pronotum, 0.73 mm.; abdomen, 1.00 mm.

Holotype, male, and *allotype,* female, Picacho Pk., Arizona, Nov. 3, 1940, R. A. Flock (in collection of U. S. National Museum). *Paratypes,* twelve males and females taken in bat caves with types; nine males and females, Tucson, Arizona, H. G. Hubbard, taken in nest of wood-rat *(Neotoma albigula)*; one male, Ft. Yuma, Arizona, H. G. Hubbard; one male and one female, Palm Springs, California, Hubbard; and one female, Indio, California, May 24, 1934, F. S. Stickney, in decaying dates (in collections of U. S. Nat. Mus., Univ. of Arizona, R. C. Flock, and authors).

The specimens taken by Hubbard bear manuscript names of Uhler, labeled as new genus and species. Fully winged individuals are desired as the unique pronotal characters may be associated in part with the partial failure of wing development.

Percy Edgar Brown

1885-1937

Agriculture and the related sciences are deeply indebted to many men who, like Percy Edgar Brown, worked diligently to discover new knowledge and train young men to "understand and to use both new and existing knowledge for the benefit of mankind."

"A man's greatest privilege is to exercise an influence which will go on after he is through work." It certainly can be stated with emphasis that Doctor Brown's influence in the fields of Soil Bacteriology, Soil Fertility, and Soil Survey will be a potent factor through the years to come, in inspiring and helping men to strive for a broader knowledge of soils and of the soils problems which must be solved if the United States and other nations are to have a profitable and enduring agriculture.

Doctor Brown was recognized as an authority in this country and abroad. He was the author of an unusually large number of scientific articles, bulletins, and special reports; he was an administrator of outstanding ability, and rendered distinguished service to agriculture and science as an officer in various state and national organizations. For a number of years he was secretary of Section "O" (Agriculture) of the American Association for the Advancement of Science, and a fellow of that Association, a fellow of the Iowa Academy of Science, and a member of the American Chemical Society. In 1920 he was elected secretary-treasurer of the American Society of Agronomy, a position he held until the time of his death except for the year 1932 when he served as president. He was elected a fellow of the Society in 1925. Doctor Brown's great contribution to that organization over a long period of years has been referred to by the editor of the Journal of the American Society of Agronomy in these words: "I feel that the American Society of Agronomy and all that it stands for today is one of the many splendid monuments that Doctor Brown has left to his enduring memory."

In 1926 he was president of the American Soil Survey Association; in 1913, Councilor for the Society of American Bacteriologists; in 1918, expert on the National Research Council; during the period 1926-36 he was Business Manager of the Iowa State College Journal of Science. He was Editor-in-Chief of the Journal at the time of his death. He was also a member of the American Organizing Committee for the First International Congress of Soil Science, and one of the twelve delegates appointed to attend that convention in Washington.

But Doctor Brown did more than these things; he had the courage and vision to insist that members of his staff and his

students maintain at all times the high ideals of scholarship and responsibility which to him were of the utmost importance. He believed in these ideals and practiced them in his daily life.

It is not surprising that a man with such a background established an enviable record as a leading educator, research worker, administrator, and author. Although a relatively young man when he finished his work, Doctor Brown left a long list of publications based for the most part on his extensive studies and researches in soil bacteriology, soil fertility, and soil management. Many of his journal articles and bulletins are notable because they make available to students of soil science new and worth-while data which will contribute in no small measure to the solution of many problems in the fields of agriculture and soil bacteriology.

W. H. STEVENSON

SOME CONTRIBUTIONS TO SOIL MICROBIOLOGY AND THEIR INFLUENCE ON THE DEVELOPMENT OF THIS SCIENCE

F. B. SMITH

From the Soils Department, University of Florida

"Soil Microbiology or Soil Bacteriology as it is called in some quarters, is a science which by its nature and status is certainly a distinct branch of science. It has many complex relationships but is most closely allied to Soil Science or Pedology as a part of the large group of agricultural sciences. It is developed from Bacteriology and Botany, from Chemistry and Geology, from Zoology and Pedology. It utilizes all these sources. But it has developed a technique all its own, it has accumulated facts, correlated and classified them, drawn deductions from them, and definitely demonstrated its place among present day sciences."—*P. E. Brown.*

It has been said that history is the biography of the world's great men. The history of the development of a science consists of the contributions to our knowledge in that field. If Soil Microbiology is considered as that branch of science which treats the micro-organisms in soils, their character, life history, and functions and their relationship to the fertility or crop-producing power of the soil, then a list of the contributions which have served to form our modern concept of the science might include, among others, titles such as "Bacterial Activities in Frozen Soils," "Micro-organisms in Some Soil Profiles in Iowa," "Effects of Manganese Salts on Ammonification and Nitrification," "Soil Inoculation With Azotobacter," "Phosphorus Assimilation by Certain Soil Molds," "The Importance of Mold Action in Soils," "Bacteriological Effects of Liming," "The Effects of Different Cropping Systems on Bacterial Action in Soils," "Bacteria at Different Depths in Some Typical Iowa Soils," and "Legume Inoculation." One need search no further to find proof in the belief that soils are dynamic, alive, and that changes are constantly taking place which may have far-reaching significance. It would be far more difficult to appraise the influence of these contributions than to list some of their implications.

A knowledge of the soil population, biological interactions, and the effects of such factors as moisture, temperature, reaction, and food supply on numbers and kinds of organisms, are topics still very much alive today. It may be inferred from these contributions that the population of the soil was expected to vary and that the findings for one soil type did not necessarily apply to all other soil types. The soil type was defined as a soil which, wherever it occurs, is relatively uniform in all its profile characteristics, *including its biological properties.* It was the placing of the investigations in soil microbiology upon such a scientific basis that led to its early recognition as a science. On the other hand, the practical side of soil microbiology was emphasized in recognizing the importance of the relation to soil fertility or the crop-producing power of the soil.

[349]

In these and other investigations reported by P. E. Brown and his associates, the whole field of the science was covered. In addition to the work on the numbers and kinds of micro-organisms in different soil types, the different horizons, and under different seasonal conditions, the effects of soil treatments, such as farm manures, fertilizers, lime, crop residues, and cropping systems, on numbers, species, activity, and the physiological efficiency of different groups were studied. Important findings on the inoculation of soil with Azotobacter and the occurrence and distribution of Azotobacter in Iowa soils were reported. The relation of nitrogen, phosphorus, and calcium in soils and in pure cultures on growth and nitrogen fixation by Azotobacter was the subject of numerous studies.

The efficiency of cultures for the inoculation of legumes was carried as a project over a number of years and unpublished reports before the name of the legume bacteria was changed to Rhizobium indicate a belief in the existence of several species of this genus. Definite experimental evidence was obtained showing physiological differences in the alfalfa, red clover, and soybean organisms. Studies on the utilization of carbon compounds by Rhizobia and the effects of fixed nitrogen, reaction of medium and the minor elements on the growth and efficiency of these organisms were made. The associative growth of legumes and nonlegumes, the nature of the symbiotic relationship between the bacteria and legumes, the mechanism of nitrogen fixation, and the form of nitrogen fixed were subjects under investigation from time to time.

The importance of mold action in soils was recognized, and as a result of the emphasis on this subject a number of contributions to our knowledge of the occurrence and action of fungi in soils were made. "A Summary of the Soil Fungi," by Gilman and Abbott, the most complete work of this nature, is one of the many contributions along this line.

In these contributions one encounters in numerous places the implication that micro-organisms, being plants, are better suited to test the fertilizer needs of soils than chemical methods. The relationship of phosphorus to the fixation of nitrogen by Azotobacter, the assimilation of phosphorus by various soil fungi and bacteria, and comparisons of chemical and biological methods were subjects investigated.

The decomposition of organic matter and the formation of humus in soils was carried as a project over a number of years, and the problem was investigated from many different angles. The chemical nature of organic matter, its reactions and relation to the mineral fraction of soil, the organisms involved, their end products, methods of measurement and rate of disappearance, the relation of organic matter to microbiological activity, soil fertility, water movement in soils, and soil conservation were studied. The influence of the carbon-nitrogen ratio of organic matter on the rate of decomposition and the fixation of carbon in soils; the influence of decomposing organic matter on the pH and the exchange capacity of the soil; the decomposition of leguminous green manures in limed and acid soils and the influence of lime on the respiratory capacity of the soil were

investigated, and reports appeared from time to time setting forth the results of these investigations. These reports have figured largely in our present concept of the importance of organic matter as a soil constituent, its transitory nature and its practical importance in soil management.

Many other phases of the science, such as nitrification, sulfofication, ferrification, denitrification, nitrate assimilation, and the isolation and identification of soil micro-organisms, were investigated, but sufficient has been said undoubtedly to show the extent of the work done, the scope of the investigations, and to indicate the practical importance ard scientific significance of the results which have been obtained.

SOIL RESPIRATION STUDIES ON THE DECOMPOSITION OF NATIVE ORGANIC MATTER[1]

WALTER B. BOLLEN[2]

From the Department of Bacteriology, Oregon Agricultural Experiment Station

"The life of microorganisms in the soil is the result of the phenomenon of assimilation in its entirety."—Stoklasa.

A physiological consequence of the assimilative activities of microorganisms in the soil is the excretion of CO_2 as a metabolic waste product. Since the food element consumed in largest proportion by the majority of soil microbes is carbon and since most of this is oxidized to yield energy, CO_2 is the outstanding product of their activity. Various intermediate products may be formed, but these are later attacked by other microorganisms and sooner or later are completely oxidized.

The various plant and animal residues which constitute soil organic matter are subject to more or less rapid decomposition according to their energy content and availability as determined by structure and composition. Wollny (43) was the first to show that practically all the CO_2 in the soil, aside from that produced by roots of higher plants, was directly due to the activity of micro-organisms in effecting this decomposition. Stoklasa (28), who did some of the first fundamental work in soil respiration, calculated that certain bacteria produce in 1 hour 2 to 2½ grams of CO_2 per 100 grams of cell substance on the dry basis; more recent data (Rahn (20)) indicates some bacteria and yeasts acting on sugars are much more active, fermenting two to three times their own weight of substrate and producing up to one-half their own weight of CO_2 per hour. Van Suchtelen (36) measured the amount of CO_2 given off by various soils under different conditions and found production of the gas to be closely proportional to numbers of bacteria present. Later investigations both here and abroad have been concerned with methods of studying CO_2 production in soils and with the amount produced from different materials and under varying conditions; these are summarized by Waksman and Starkey (41). Soil respiration studies have thus attained importance as a measure of decomposition processes and as an index of soil fertility.

A supply of rapidly decaying organic matter in the soil insures desirable microbial activity, greater availability of nitrogen and other plant food elements, and the maintenance of good tilth. Since these consequences mean better crop yields and since decomposition results in loss of organic matter, a permanent system of soil fertility must include practices by which crop residues or manures are regularly added to the soil.

Numerous investigations (16) have shown that crop yields closely correlate with the organic matter content of the soil. It is also well established that the rate of loss of organic matter is influenced by inherent soil properties, environmental factors, and any treatment which affects microbial action in the soil. Hence, a study of soil organic matter and its rate of decomposition is of great practical importance.

OBJECT OF INVESTIGATION

A series of studies on CO_2 evolution from various soils, with and without the addition of organic materials and supplemental treatments, have been in progress at this station for a number of years. These have been complemented by chemical and microbial analyses made on the original soil samples and on the soils from the respiration apparatus during and at the close of the incubation period. In this way it was hoped to gain information on the effect of factors, both artificially impressed and inherently fixed by soil type, on the rate of humus formation, accumulation and decomposition. Data from these investigations have been segregated and some are here presented as a preliminary report showing the rate of decomposition of the native organic matter of certain similar and dissimilar soil types occurring in Oregon and elsewhere along the Pacific coast, and indicating the influence of moisture and available nitrogen, factors more or less subject to practical control.

SOILS STUDIED

Soils from distinct taxonomic groups as well as similar soils within a group were included in these studies. The ones selected include seven acid soils from the Willamette Valley, two alkaline soils from California, and one "alkali" soil from eastern Oregon. Samples, except where otherwise noted, were taken along fences or from other areas approaching virgin soil conditions as nearly as possible and were taken to a depth of 6 inches after removing surface litter or sod. Each sample was passed through a 2-mm. screen, mixed well, and stored in a large can for one to two weeks before setting up the respiration experiment.

ACID SOILS

All the acid soils were obtained in the Willamette Valley and are pedalfers representative of several groups developed under a mild subhumid climate from alluvial valley-filling materials derived mainly from basaltic rocks. They are noncalcareous, faintly to definitely acid, and range in texture from light to heavy. Their important pedologic characters are presented in the following outline:

RECENT SOILS: friable soils on friable subsoils, with little profile modification.

1. *Newberg loam:* This is a first bottom soil, formed in swift water, with coarse subsoil. It is a brown mellow loam averaging 12 inches in depth on a brownish sandy loam subsoil. Under virgin con-

ditions the soil contains a moderate supply of organic matter, but under cultivation this is soon lost. The soil is subject to overflow and reworking by high water and has a ridged or billowy relief. Drainage is good to excessive, and the soil dries out quickly. It is a productive soil but applications of manure are especially valuable because of the effect in increasing moisture-holding capacity.

2. *Chehalis loam, and*

3. *Chehalis sandy loam:* These are second bottom soils formed in backwater, with heavier subsoil. They are brown mellow soils 9 to 12 inches deep on stratified subsoil which, to a depth of about 4 feet, is the same or slightly heavier in texture and somewhat lighter in color than the surface soil. The soil is subject to overflow almost every year, but drainage takes place rapidly after the backwater subsides. A relatively low supply of organic matter is present.

OLD VALLEY FILLING SOILS: Mature soils with compact subsoils.

4. *Willamette silty clay loam:* This soil consists of maturely weathered, old valley-filling deposits which have developed under good drainage. The surface soil has an upper layer, 1 or 2 inches thick, of brown, loose granular loam containing organic matter in varying stages of decomposition. The subsurface layer, extending to a depth of about 10 inches, consists of a dark brown, firm, silty clay loam which breaks into fine clods when dry. The surface soil is leached of readily soluble minerals and colloids, which have infiltrated into the subsoil, and it is moderately acid. To a depth of from 30 to 35 inches, the upper subsoils consist of a vesicular, heavy silty clay loam; this grades into the lower, denser subsoil which, at approximately 4 feet, contacts dense, slightly compact, yellowish clay loam or silty clay loam parent material of varying thickness overlying gravel deposits.

5. *Sifton gravelly fine sandy loam:* This is a black-brown prairie island soil with a strongly developed horizon of humus accumulation; it differs from the true chernozems in lacking a horizon of carbonate accumulation. Soils of the Sifton series are high in finely divided, partly decomposed organic matter and are popularly termed "loose land." They are very dark brown or black when moist. When the dry soil is cultivated a cloud of dust which settles as a sooty deposit is formed. The surface soil to a depth of approximately 15 inches is a dark brown, loose fine sandy loam containing gravel ranging to 2 inches in diameter. Nearly 30 per cent of the sample collected for this work would not pass a 2-mm. screen. The subsoil, which extends to about 3 feet, is similar to the surface soil but contains less organic matter and becomes grayish brown in color. Underlying this is a highly porous and gravelly fluvio-glacial deposit of parent material.

6. *Dayton silty clay loam:* To a depth of 8 or 10 inches this soil is a dull brownish-gray, slightly compact silty clay loam. The area sampled was covered by sod, and the first 2 inches were dark gray

and somewhat granulated; below this the remainder of the A horizon progressively lightened in color due to increasing penetration of podzolic flour. Plowed areas of this soil when dry have a dingy white appearance and it is locally called "white land." Minute ortstein concretions are also distributed in the A subhorizon. The subsoil, extending to about 3 feet, consists of bluish-gray plastic impervious clay irregularly mottled with yellowish-brown clay loam. Soils of the Dayton series are derived from heavy compact alluvial clay and silt, have weathered under poor drainage, and are strongly acid. Nikiforoff (14) describes them as glei-meadow podzols. They contain a moderate supply of organic matter, decomposition of which is retarded by a water-logged condition during the winter and spring rainy seasons and by an extremely dry compact condition during the summer.

HILL SOILS: residual.

7. *Aiken silty clay loam:* This is a red podzolic soil of lateritic derivation. To a depth of 10 inches the surface soil consists of a red silty clay loam; it is granular in structure, and contains numerous iron-cemented pellets. The surface, 1 to 1½ inches, is tinged with brown due to an appreciable content of organic matter. The subsoil is a less silty clay, slightly redder in color; it is compact and dense when moist but breaks down to a coarse cloddy structure when dry. Extending upward into the lower subsoil from the parent basaltic bedrock, which occurs at about 5 feet, are many partly weathered, angular fragments which produce rust-brown mottles. Although the soil is sticky when wet, it is friable and easily cultivated when the moisture content is favorable.

ALKALINE SOILS

Two slightly alkaline soils were obtained from a citrus grove in the San Joaquin Valley. These had been under cultivation for a period of years without addition of organic manures.

8. *San Joaquin sandy loam:* This is a secondary pedalferic soil formed from transported material of granitic origin under semiarid conditions. It has a mature profile with well-developed hardpan at 28 to 30 inches. The surface soil, about 6 inches deep, is a dark reddish-brown sandy loam high in colloidal material and poor in organic matter. It is decidedly sticky when wet, but the presence of coarse gritty fragments and mica particles mask plasticity. The subsoil is a red heavy clay; it is gritty with coarse angular fragments and sand, but a high colloidal content exerts predominating influence on structure and consistence. The soil is definitely basic in reaction but noncalcareous.

9. *Porterville clay adobe:* Soils of the Porterville series are secondary pedocals formed under semiarid climate from transported material derived primarily from basic igneous rocks. The parent material was largely soil eroded from hillsides and washed to its present position. The midmature profiles developed under semiarid

climate and are characterized by calcareous concentrations. In the area sampled, the surface soil, 6 inches deep, is chocolate-brown, heavy in texture, and has a typical adobe structure. On drying, the adobe blocks break by secondary cracking into small angular granules to a depth of several inches; this results in an unstable structure, forming what is locally termed a "dry-bog." It is readily puddled by working when wet. The reaction is slightly basic and the organic matter content is relatively low. The subsoil is similar to the surface soil in color and texture but contains light streaks and stains due to lime accumulation which increases with the depth to form segregated plates and lens. Drainage is poor. The soil is so impervious that once saturated it dries very slowly.

"ALKALI" SOIL

10. *Vale heavy loam*[3]: The "alkali" soil, obtained from the Vale Branch Experiment Station in eastern Oregon, is a strongly salinized heavy loam, dispersed and moderately compact. The native vegetation is chiefly greasewood *(Sarcobatus vermiculatus)*, sage brush *(Artemisia tridentata)*, and salt grass *(Distichlis spicata)*. From 0 to 5 inches the profile consists of grayish-brown dispersed loam that is sticky when wet but friable under proper moisture conditions. The 5 to 20-inch horizon is similar in texture but lighter in color; when dry the upper 5 inches exhibits columnar structure which grades to a nut structure below. From 20 to 40 inches is indurated calcareous alkaline heavy loam to clay loam pan. Below this is light yellow-brown, more friable material streaked with sand; this rests on parent sand and gravel alluvium derived from the Payette formation. The soil developed under a hot arid climate and is heavily salinized with native alkali salts. An analysis of the sample gave the following results:

WATER SOLUBLE SALTS	PPM
Total	3,150
CO_3	1,019
HCO_3	0
S as SO_4	79
Cl	108
Na	1,200
Ca	76

REPLACEABLE BASES	EQUIV. PPM
Exchange capacity	242
Ca and Mg	25
Na	203
K	15

The reaction is strongly alkaline (pH = 9.8), and the organic content is low.

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[3] This soil has not been mapped by the Soil Survey Division, and the name here used is not official.

TABLE 1
Chemical Analyses of Soils Studied*

Soil	Organic Matter	Kjeldahl Nitrogen	C:N†	pH		S as SO₄		N as NO₃§		P as PO₄	
	Percentage	Percentage		Original	Incubated‡	Original	Incubated	Original	Incubated	Original	Incubated
	Percentage	Percentage				ppm	ppm	ppm	ppm	ppm	ppm
1. Newberg loam	2.35	0.15	9.0	5.7	5.8	2	2	8	28	0.0	1.0
2. Chehalis loam	1.65	0.10	9.6	6.8	6.7	0	0	11	55	0.2	2.1
3. Chehalis sandy loam	0.99	0.06	9.6	6.6	6.4	1	25	37	32	0.7	0.1
4. Willamette silty clay loam	3.71	0.23	9.3	5.9	5.9	2	0	19	45	0.3	2.1
5. Sifton gravelly fine sandy loam	9.55	0.63	8.8	5.3	5.0	1	0	28	110	0.0	0.0
6. Dayton silty clay loam	3.24	0.18	10.4	5.6	5.5	7	30	55	38	3.5	1.3
7. Aiken silty clay loam	4.51	0.19	13.7	6.0	5.7	1	27	70	63	0.5	0.1
8. San Joaquin sandy loam	0.51	0.04	7.4	8.4	8.0		16	143	36	1.5	1.3
9. Porterville clay adobe	1.58	0.15	6.1	8.1	8.2	31	29	81	72	0.3	1.4
10. Vale heavy loam	1.31	0.05	15.2	9.8	9.6	79	83	2	5	0.1	0.1

* Data expressed on basis of water-free soil.

† Carbon calculated from organic matter, using C = organic matter ÷ 1.724.

‡ "Incubated" refers to samples removed from respiration apparatus at close of the respiration period.

§ N as NO₂ was also determined; in all cases the concentration was less than 1 ppm.

It is of interest to compare this soil with the two alkaline soils from California. The data for organic matter and C:N ratio'in Table 1 show that while the Vale soil is low in total nitrogen and in organic matter, it has the widest C:N ratio; the California soils, also low in organic matter and total nitrogen, have the narrowest C:N ratios. All three are semi-arid soils developed under similar moisture conditions, with an average annual precipitation of 8 to 9 inches, much of which occurs during the winter months. At Vale the average temperature during November to March is 30° F.; at Porterville, in the San Joaquin Valley, the average during the same period is 50° F. In the latter locality climatic conditions thus favor a rapid and extensive decomposition; in the Vale region low temperature inhibits decomposition when moisture is favorable, while during warmer periods moisture is limiting. Thus is expressed an outstanding influence of climate on soil organic matter through its influence on "living nature." (12) This influence is further reflected on the rate of CO_2 evolution when optimum conditions are established, as is shown later.

METHODS

Duplicate 1-kg. portions of each soil, on the water-free basis, were placed in jars of a respiration apparatus similar to that of Potter and Snyder (17). The moisture content of the soils was adjusted to 50 per cent of the saturation capacity: of the required amount of water a portion, insufficient to cause danger of puddling, was mixed with the soil before transferring it to a jar; the soil was then placed in the jar and caused to settle as uniformly as possible by tapping the jar on the table, after which the remainder of the water was poured on around the edge. Slight pressure rather than suction was used to pass CO_2-free air over the soil; the pressure did not exceed that necessary to force the air through a 4-inch column of approximately N/1 NaOH used as absorbent in a test tube. This procedure has the advantage of maintaining the pressure over the soil as well as over the absorbing solution at approximately normal atmospheric pressure. The apparatus was incubated at room temperature, which, during the course of the various experiments, showed an extreme variation of 20° to 32° C. but generally ranged with ± 2° of 23° C. as indicated by a recording thermograph. The greater variations in almost every case were not more than a few hours in duration, and their temporary influences can be disregarded in long-continued experiments. Absorbent was changed at daily intervals during the first 5 days, then at gradually increasing periods until the final determination, which was made 30 days after the one preceding. The absorbed CO_2 was determined by double titration, using thymol-blue and brom-phenol-blue as recommended by Little and Durand (10). Results are expressed in milligrams of carbon as CO_2 evolved per kilogram of soil. The incubation period of the experiments from which data are given varied from 91 to 115 days; the curves of Figures 1 and 4 have been arbitrarily extrapolated or shortened as required to 100 days.
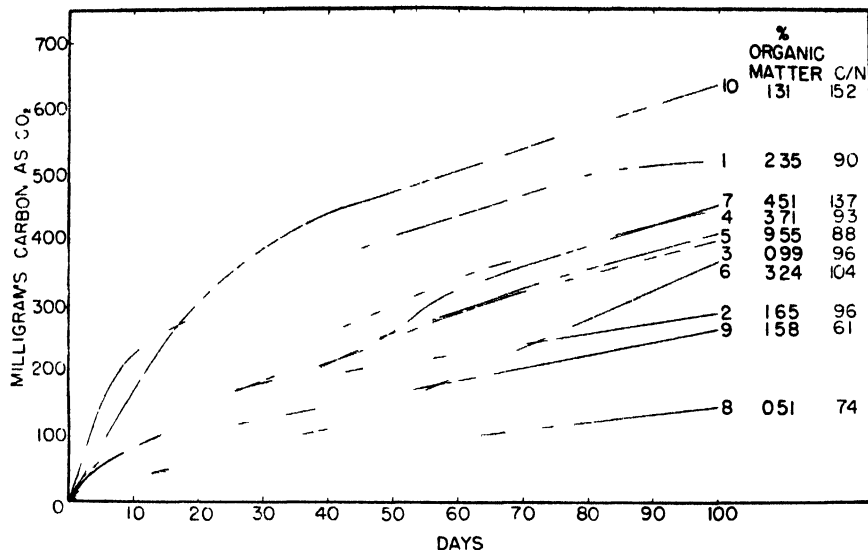
FIG. 1. Decomposition of Native Organic Matter in Different Soils. 1—Newberg loam, 2—Chehalis loam, 3—Chehalis sandy loam, 4—Willamette silty clay loam, 5—Sifton gravelly fine sandy loam, 6—Dayton silty clay loam, 7—Aiken silty clay loam, 8—San Joaquin sandy loam, 9—Porterville clay adobe, 10—Vale heavy loam.

Moisture was determined by heating samples to constant weight at 105° C. Saturation capacity was determined by placing the soil in gooches, flooding from below by immersing almost to the rim in water, and allowing free drainage to occur in a saturated atmosphere; as soon as free water failed to appear within 2 minutes on the bottom of a draining gooch after wiping dry, it was heated to constant weight at 105° C.; from the weight of the dried soil and the weight of water lost, the percentage of water held by the soil was calculated on the water-free basis.

Chemical and microbial analyses were made as follows:

A 1:5 suspension of the soil was prepared with sterile distilled water and shaken for 30 minutes; the coarser particles were then allowed to settle, and a 1-cc. sample was withdrawn for the preparation of dilutions for plate counts. Determination of pH, by means of the circulating hydrogen electrode (2), was made on another portion. The remaining suspension was then filtered, and the filtrate was analyzed. Association of Official Agricultural Chemists methods of analysis for waters were used for the determination of nitrate and nitrite. Sulfate was determined by the turbidity method of Schreiner and Failyer (24). Truog and Meyer's modification (31) of the Deniges method for phosphate was used.

For the microbial counts, peptone-glucose-acid agar was used for molds, and sodium albuminate agar, as described by Fred and Waksman (5), was used for bacteria and actinomyces. Duplicate plates from appropriate dilutions were poured in 150-mm. petri dishes; the number of colonies counted after incubation at 28° C. ranged from approximately

50 to 250. Numerous control plates, amounting to at least 10 per cent of the total poured, were used. Azotobacter were determined by the soil-paste plaque method, using 5 per cent starch and 1 per cent powdered $CaCO_3$.

The original soil samples were analyzed for organic matter by Rather's (21) method, and for total nitrogen by the Kjeldahl procedure (1).

Analytical results are given in Tables 1 and 2. At the close of the experiment samples of each soil were removed from the respiration apparatus and analyzed for micro-organisms, water-soluble anions, and pH; these results provide more direct comparison of the various soils since each had been maintained at an equivalent moisture percentage on the basis of water capacity. Only soil No. 6 lost an appreciable amount of moisture, 2.9 per cent, during the incubation period.

All data presented are averages of duplicate determinations which checked reasonably in each instance.

DECOMPOSITION OF NATIVE ORGANIC MATTER

Few previous studies on respiration of the soil's own organic matter have been reported. Isaac (7), using respiration periods of not over sixteen days, found that geological character of parent material was a definite though minor factor in determining differences in respiratory power of different soils; different types of vegetative covering appeared to exert a greater modifying effect. Vandecaveye (34), in a more extended experiment, observed greater CO_2 production in Palouse silt loam than in Helmer silt loam, two soils developed from identical parent materials under similar climate but under different vegetation. Data on untreated soils included as controls in experiments set up to study decomposition of added organic matter are available from numerous reports, but since the soils in most cases are incompletely described, particularly as concerns moisture capacity, the results are not directly comparable on the desired basis.

The results presented in Figure 1 show that under like temperature and equivalent moisture conditions the amount of CO_2 evolved from decomposition of native organic matter is determined largely by three factors: (1) the amount of organic matter present, (2) the soil texture, (3) the C:N ratio. The influence of the first factor is obvious. Production of CO_2 is not, however, proportional to carbon content (41). The amount of organic matter, as well as its nitrogen content, depend largely upon climate, while vegetation, topography, parent material, and age are influential in the order given (8). Texture is important because aeration increases as the square of particle size. The importance of the C:N ratio follows from nutritional requirements of organisms active in the decomposition; next to carbon, and aside from oxygen and hydrogen which are always abundantly supplied by organic matter and water, the food element required in largest amount is nitrogen. Hence, an insufficiency of avail-

able nitrogen limits microbial development, and nitrogen is most commonly the limiting food element.

The C: N ratio of humus as well as of fresh organic matter controls to a great degree the rate of mineralization and liberation of plant nutrients (26, 37, 38). In fresh vegetable material the ratio ranges from about 80:1 for nonleguminous to 40:1 for leguminous plants; the former will not be rapidly decomposed unless additional nitrogen is available in the soil, whereas leguminous material contains more nitrogen than required by the micro-organisms active in the decomposition, and the excess will be liberated as ammonia. Humus in surface soils has a C:N ratio approximating 10:1 on the average, but ratios of 3.5:1 to 35:1 have been reported (9). A wide ratio, such as 20:1, indicates the organic matter has not
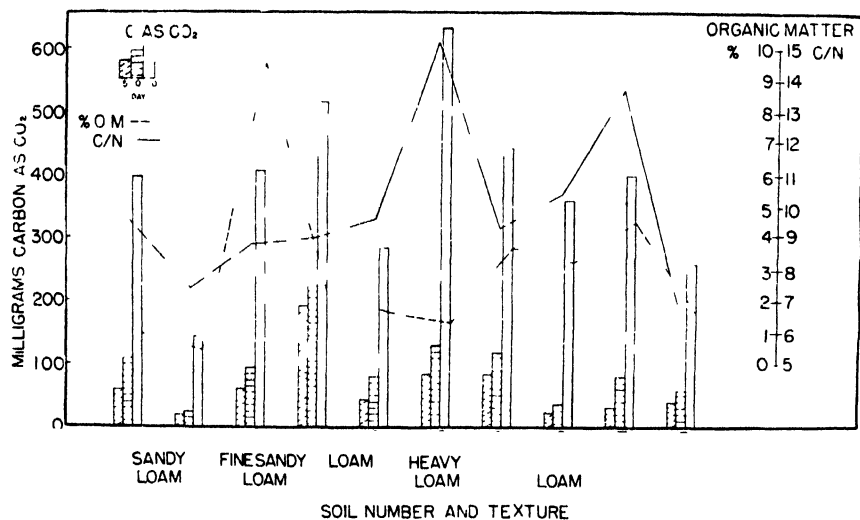
Fig. 2. Relation of Organic Matter, C:N Ratio, and Texture to Soil Respiration. 1—Newberg loam, 2—Chehalis loam, 3—Chehalis sandy loam, 4—Willamette silty clay loam, 5—Sifton gravelly fine sandy loam, 6—Dayton silty clay loam, 7—Aiken silty clay loam, 8—San Joaquin sandy loam, 9—Porterville clay adobe, 10— Vale heavy loam.

undergone extensive decomposition and that it can still support rapid development of micro-organisms under favorable soil conditions. A ratio of 10:1 or less indicates an advanced stage of decomposition; such residual material is resistant and is subject to attack only by the autochthonous microflora (4, 42), which is physiologically adapted to its utilization, and further decomposition is slow.

In Figure 2 is shown graphically the relation of organic matter, C:N ratio, and texture to CO_2 evolution in the soils studied. The San Joaquin sandy loam contained the least amount of organic matter as well as a very narrow C:N ratio and gave the lowest CO_2 evolution. The lowest C:N ratio occurred in the Porterville clay adobe soil. This soil contained three times as much organic matter as the San Joaquin sandy loam and, despite

a narrower C:N ratio and its heavy texture, gave off about twice as much CO_2; all the other soils, having wider C:N ratios, ranked above it. Sifton soil, with by far the highest organic matter content, also had a relatively low C:N ratio and was only intermediate in respiratory power. In contrast to these soils, the Vale heavy loam, next to the lowest in organic matter but with the widest C:N ratio, gave the greatest CO_2 production; as was shown in a previous paper (3). CO_2 from mineral sources contributed only 10 per cent of the total evolved, an amount insufficient to change the relative position of this soil in respiratory intensity. In these four soils, which present an extreme range in texture, in supply of organic matter, and in C:N ratio, the dominant factor in controlling respiration appears to be the C:N ratio.

Respiration in Aiken silty clay loam soil showed a response correlating with a comparatively high organic content and a wide C:N ratio. Texture is the dominant factor in Newberg loam. For the remaining soils, predominating influence can hardly be ascribed to any of the three factors.

The order of the various soils according to respiratory power varied little during the course of incubation. Figure 1 shows the soils which were high or low during the first few days were also high or low at the close. There is some crossing of the curves; in general, the shifts involved only two closely ranking soils. At the beginning the rate of CO_2 production was relatively greater in the coarser soils. Later, it dropped in these soils and increased slightly in the heavier soils. This is emphasized in Figure 2.

The microbial counts on the whole show relatively small variations between the different soils before, as well as after, the prolonged incubation. In the original samples the number of molds correlated to some extent with the organic matter. With few exceptions the bacteria and actinomyces were relatively constant throughout. In field soils bacterial numbers and nitrate production correlate closely with CO_2 evolution, as has been shown by the pioneer work of Russell and Appleyard (22, 23) and by numerous more recent experiments. Under uniform conditions in the laboratory the most extensive changes take place during the first few weeks; after prolonged incubation the soil tends to return to an equilibrium similar to the original condition (17, 41). Data in Tables 1 and 2 show this tendency. Unfortunately, no analyses were made during the incubation period.

Nitrates showed appreciable increase or slight decrease in most cases. In Sifton soil a large increase correlating with organic content was obtained. In San Joaquin sandy loam, which was lowest in total nitrogen and organic matter, the exceptionally high nitrate content became markedly lowered during incubation; there is no apparent correlation of this change with the other data. Nitrate accumulation in Vale heavy loam was negligible, possibly due to the wide C:N ratio or to high alkalinity, which inhibits nitrifying bacteria (27).

Changes in pH were comparatively small. In most cases it decreased. In the "alkali" soil the decrease of 0.2 pH was accompanied by a 40 per

TABLE 2
Moisture Relations and Microbial Analyses of Soils Studied*

Soil	Water Capacity	Moisture		Molds		Bacteria		Actinomycetes		Azotobacter‡	
		Original	Incubated†	Original	Incubated	Original	Incubated	Original	Incubated	Original	Incubated
	Percentage	Percentage	Percentage	Thous.	Thous.	Millions	Millions	Millions	Millions		
1. Newberg loam	60.0	20.1	30.2	38	39	5.1	4.8	0.6	0.6	+++	+++
2. Chehalis loam	40.0	19.0	20.2	55	70	2.1	4.9	0.5	0.6	+++	+++
3. Chehalis sandy loam	36.2	4.6	18.0	59	20	1.7	2.3	0.3	0.6	++	++
4. Willamette silty clay loam	54.2	27.2	25.5	56	24	3.4	1.4	2.6	2.2	++	++
5. Sifton gravelly fine sandy loam	98.0	31.1	48.8	365	43	4.7	2.5	2.9	0.7	0	0
6. Dayton silty clay loam	50.8	8.5	22.5	57	73	2.2	3.8	0.6	2.7	+++	+++
7. Aiken silty clay loam	54.2	17.1	26.8	52	37	0.9	0.9	0.2	6.0	++	+++
8. San Joaquin sandy loam	15.0	4.6	7.3	13	20	9.3	3.3	1.3	1.8	++++	++
9. Porterville clay adobe	45.2	10.3	27.4	13	70	3.7	6.3	0.8	1.7	+++	++
10. Vale heavy loam	57.0	17.6	28.1	10	20	1.4	2.0	0.2	0.6	0	++

* Data expressed on basis of moisture-free soil.
† "Incubated" refers to samples removed from respiration apparatus at close of the respiration period.
‡ Development on soil-starch plaques; O = no colonies; + = few colonies, ++ + = many colonies.

cent decrease in concentration of normal carbonate. Since hydrogen-ion concentration is an important factor controlling activities, it follows that CO_2 production should reflect this influence. Various reports have shown this to be so (28, 30, 35), but other factors may have predominating and masking influence, as in the present study.

Sulfates and water-soluble phosphate showed increases as well as decreases, but there is no consistent correlation with organic matter or CO_2 production.

EFFECT OF MOISTURE

Moisture is one of the environmental factors controlling development of micro-organisms. As available water in the medium falls below a certain minimum, diffusion is retarded, growth is slackened, and finally, the organism assumes a dormant or spore stage which retains life for a longer or shorter period characteristic of the species under the environment as fixed by temperature, pH, and other factors. Above some maximum percentage of water the physiology of the organism will be affected chiefly by the resultant effects on free oxygen supply; in soil layers of appreciable thickness, saturation with water retards or eliminates aerobic action and results in a preponderance of anaerobic and facultative species. The controlling factors, as pointed out by Rahn (19), are aeration and thickness of moisture film. Optimum aeration and optimum thickness of moisture film cannot co-exist in arable soils, since the particle size is so small that an optimum film, from the standpoint of diffusion rate, is attained only in the water-logged state. As particle size decreases, thickness of capillary films decrease, although moisture capacity increases. The moisture capacity of soils varies not only with texture, but also with structure and organic matter content. For this reason the optimum soil moisture for a particular species or association of species varies with different soils, even those of similar texture. For general soil microbial activity, the optimum is commonly considered to be that amount of moisture present when the soil is approximately 50 per cent saturated.

Since the CO_2 evolved in soil respiration results from the combined activities of all microbes present, anaerobes as well as aerobes, and autotrophes as well as heterotrophes, and since each kind possesses characteristic respiratory powers, it is evident that the optimum moisture for this general microbial function is determined by a complexity of interrelationships. While such definite functions as ammonification, nitrification, and nitrogen fixation have been found to have definite moisture optima (60, 60, 70 per cent, respectively (6)), an optimum range rather than an optimum point would seem more likely to apply to CO_2 evolution. Within this range a change in moisture content might increase respiratory action of some species while decreasing that of others.

Early studies showing the influence of moisture on CO_2 evolution are summarized by Löhnis (11). Stoklasa (29) found CO_2 production of a soil to be increased by increasing the moisture to 50 per cent of the satura-

tion capacity. Waksman and Starkey (41), for the optimum in carbon dioxide evolution studies, added water to bring the soil moisture to 50 per cent of the moisture-holding capacity. Vandecaveye (32) adjusted moisture to the normal field capacity as expressed by Shaw (25). With an arid soil Oberholzer (15) found that the rate at which added organic matter decomposed increased with increasing moisture up to almost complete saturation.

To study the effect of moisture on decomposition of native soil organic matter, Chehalis loam and Sifton gravelly fine sandy loam were selected
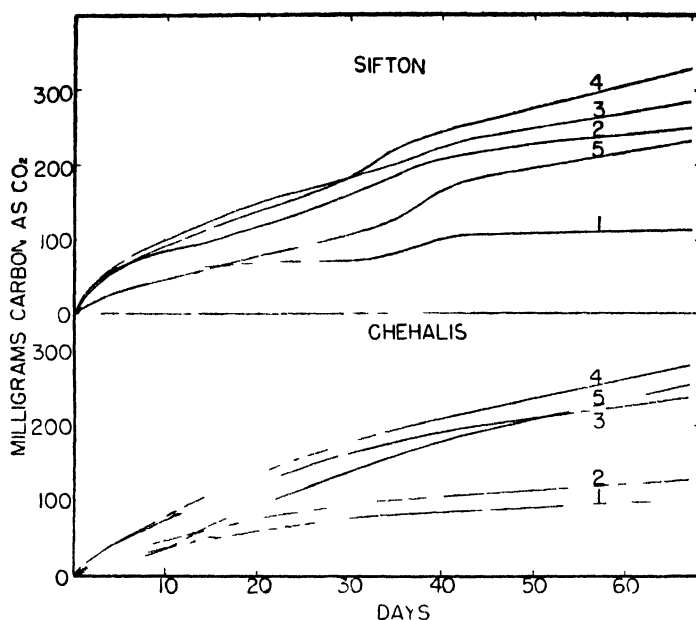
FIG. 3. Effect of Moisture on Soil Respiration.
Sifton gravelly fine sandy loam: 1—Wilting point, 2—25% saturation, 3—50% saturation, 4—75% saturation, 5—100% saturation.
Chehalis loam: 1—Wilting point, 2—25% saturation, 3—50% saturation, 4—75% saturation, 5—100% saturation.

because, while not greatly different in texture, they differ widely in moisture capacity and wilting point.[4] Moisture capacity was determined by the gooch procedure previously described, and wilting point was determined by the sunflower seedling method (44). The respective values for Chehalis loam were found to be 8 per cent and 40 per cent; for Sifton gravelly fine sandy loam, the values were 14 per cent and 98 per cent. The moisture in 1-kilogram portions of the soils was adjusted to the wilting point, 25 per cent, 75 per cent, and 100 per cent saturation . The respiration study, using the methods previously described, was carried on

[4] William H. Tomsheck, graduate student in soils, assisted in obtaining the data presented in this section.

for 68 days. After 30 days, 200 grams of soil were removed from each bottle for chemical and microbial analyses; subsequent CO_2 production in the remaining 800 grams was calculated to the original basis of 1 kilogram of water-free soil. Disturbance of the soil in the apparatus due to removal of the sample had a marked effect in temporarily increasing the rate of CO_2 evolution in the Sifton gravelly fine sandy loam, but not in Chehalis loam. This effect was probably due to both the higher organic matter content and the higher moisture capacity, which bears an inverse relation to aeration, of the Sifton soil. The effect of increased aeration during preliminary preparation of the soil is similarly expressed by the rate of respiration during the first 10 days, as may be seen from Figure 3; here, also, Chehalis loam showed least response.

In both soils CO_2 evolution increased as moisture was increased up to and including 75 per cent of the saturation capacity. Slowest respiration occurred with moisture at the wilting point except during the first 10 days, when respiration was lowest in the saturated soils. The subsequent increase in CO_2 from the saturated Chehalis loam was due to an increase in rate, indicating an adjustment or change in the microflora; the curve for saturated Sifton soil, on the other hand, gained ascendancy because the driest soil soon fell in rate of respiration. Saturated Chehalis loam soon approached and then paralleled the same soil with moisture at three-fourths saturation, which was optimum throughout. For the Sifton soil one-half saturation was optimum until the disturbance produced by sampling at 30 days; after this the 75 per cent saturated soil was superior. Differences between the soils at 50 per cent and 75 per cent saturation were not appreciable till after the first 10 days. While differences in moisture produced similar response in both soils, the effects were quantitatively greater in the soil with greater water capacity.

On the basis of these results optimum moisture for CO_2 evolution in prolonged respiration experiments is near 75 per cent of the saturation capacity.

From the results of analyses made at 30 and 60 days, only data for nitrates, total C as CO_2 evolved, and pH are given (Table 3).

Nitrate production did not correlate with CO_2 evolution at 30 days but was in fair agreement at 68 days. Since nitrification is an autotrophic transformation, consuming rather than liberating CO_2 in primary metabolism, and since it represents only a small portion of the total soil microbial activity, it can exert *per se* only a negligible influence on soil respiration. The effect of moisture on the process, which is aerobic, is likely to be indirect insofar as it controls aeration. The data show this to be true in the extreme case of complete saturation; denitrification was complete in 30 days, but CO_2 evolution was vigorous. At the same time there was little difference in the amounts of nitrate produced in either soil at 25, 50, and 75 per cent saturation. Nitrates increase with moisture up to 75 per cent saturation at the 68-day analysis. Anomalous results were obtained with both soils at the wilting point.

TABLE 3

INFLUENCE OF MOISTURE ON NITRATES AND CO$_2$ EVOLUTION*

SOIL AND MOISTURE CONTENT	N as NO$_3$		TOTAL C as CO$_2$ EVOLVED		pH	
	30 days	68 days	30 days	68 days	30 days	68 days
	ppm	*ppm*	*mg.*	*mg.*		
Chehalis loam						
Moisture at 8% = wilting point	11†	30	75	101	6.8†	6.6
Moisture at 10% = 25% saturation	54	31	89	127	6.6	6.7
Moisture at 20% = 50% saturation	34	53	156	236	6.7	6.7
Moisture at 30% = 75% saturation	44	55	176	276	6.6	6.6
Moisture at 40% = 100% saturation	47	0	127	250	6.7	7.3
Sifton gravelly fine sandy loam						
Moisture at 14.0% = wilting point	28†	125	71	128	5.3†	5.2
Moisture at 24.5% = 25% saturation	27	94	165	273	5.3	5.1
Moisture at 49.0% = 50% saturation	69	107	183	321	5.2	5.0
Moisture at 73.5% = 75% saturation	62	139	187	380	5.1	5.0
Moisture at 98.0% = 100% saturation	58	0	107	316	5.9	5.8

* Data expressed on basis of moisture-free soil
† In original sample.

The plate counts for micro-organisms in Chehalis loam showed considerable irregularity. The maximum number of molds at both 30 and 68 days occurred in the half-saturated soil. Bacteria and actinomyces were highest in 25 per cent saturated soil at both times. Numbers of molds, bacteria, and actinomyces were considerably less for all other treatments and did not fluctuate greatly; at 68 days all counts were generally higher. There was little difference between numbers in saturated soil and soil at the wilting point.

Sifton soil gave higher mold counts than Chehalis loam, but the bacteria and actinomyces in most cases were lower. The moisture content giving maximum numbers in this soil was 75 per cent of the saturation capacity; this was true at both 30 and 68 days. In neither soil was the influence of moisture on microbial numbers, as revealed by plate counts, outstanding.

Of the other analytical data obtained in connection with the moisture experiment, only the pH values merit mention. Only in the saturated soils did changes of more than 0.2 pH occur. Saturation of Chehalis loam changed the original pH 6.8 to pH 7.3 in 30 days; the same value obtained at 68 days. Saturated Sifton soil changed from pH 5.3 to 5.9 in 30 days to pH 5.8 at the close of the experiment. These decreases in acidity may have been due to ammonia accumulation, inasmuch as nitrification was inhibited by complete saturation.

EFFECT OF AVAILABLE NITROGEN

The effect of various added nitrogen compounds on decomposition of native organic matter in four soils was studied by the respiration procedure previously outlined. Curves showing the effects on CO_2 evolution are given in Figure 4. In plotting results, data for the control were subtracted from the corresponding data for the treated soil in all cases. The control is thus plotted as a straight line at zero; curves above the line indicate stimulation, while those below show a depression.

A slight stimulation from nitrogen added as ammonium sulfate was obtained in Chehalis sandy loam and Vale heavy loam. This effect persisted for 10 days in the former and about 25 days in the latter, after which the respiration intensity dropped in comparison with the controls. The response of the Vale soil correlates with its wide C:N ratio (Table 1); addition of available nitrogen permitted greater microbial development at the expense of excess available carbon, for reasons discussed in the previous section. In Chehalis sandy loam the C:N ratio is normal, but the supply of organic matter is low; apparently the nitrogen of the humus compounds in this soil were less available than the carbon, so that added nitrogen enabled more micro-organisms to grow.

With Newberg loam, striking results were obtained with urea and with sulfamic acid (HSO_3NH_2). The curve for the urea-treated soil, however, includes the additional carbon as CO_2 evolved from its own decomposition, so that any stimulation due to the nitrogen is indicated only by

an increase over this amount. Urea is rapidly hydrolyzed by a variety of soil bacteria (39) to ammonium carbonate, which decomposes to ammonia, carbon dioxide, and water. This ammonification is much more rapid than the ensuing nitrification. Analyses made on the urea-treated soil during respiration showed that while the urea was 93 per cent nitrified in 90 days it was completely ammonified in 10 days. By calculation, the amount of carbon as CO_2 liberated by ammonification of 1 gram of urea is 200 mg. The highest point on the curve (No. 8, Figure 4) occurred at 10 days; the
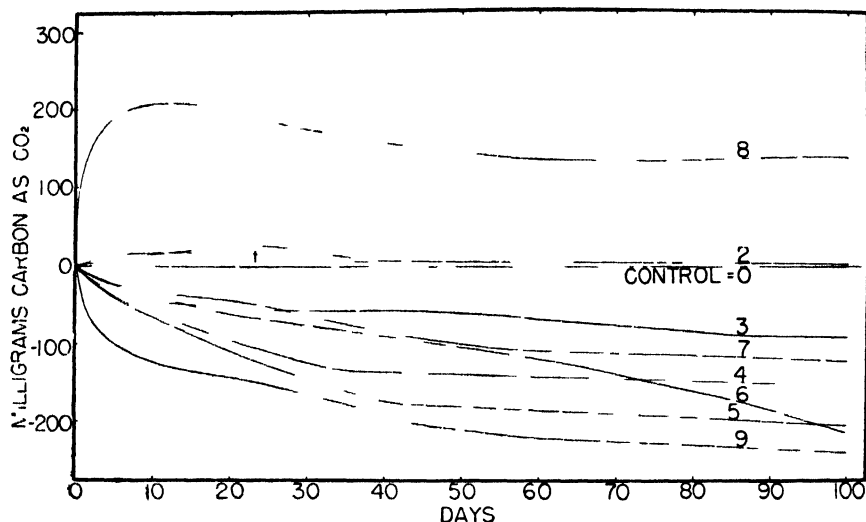
FIG. 4. Effect of Added Available Nitrogen on Decomposition of Native Organic Matter. 1—Chehalis loam + $(NH_4)_2SO_4$ equivalent to 21 ppm N. (Discontinued at 40 days), 2—Vale heavy loam + $(NH_4)_2SO_4$ equivalent to 64 ppm N , 3—Willamette silty clay loam + $(NH_4)_2SO_4$ equivalent to 106 ppm N., 4—Willamette silty clay loam + $(NH_4)_2SO_4$ equivalent to 250 ppm N., 5—Willamette silty clay loam + KNO_3 equivalent to 250 ppm N., 6—Newberg loam + $Ca(NO_3)_2$ equivalent to 150 ppm N., 7—Newberg loam + $(NH_4)_2SO_4$ equivalent to 300 ppm N , 8—Newberg loam + urea equivalent to 467 ppm N., 9—Newberg loam + HSO_3NH_4 equivalent to 433 ppm N.

reading of 208, minus 200 from decomposition of the urea, leaves a net increase over the control of only 8 mg. of carbon as CO_2 due to the added nitrogen. While this is within the experimental error it is nevertheless significant that urea caused no depression in respiration in this soil as did ammonium sulfate, calcium nitrate, and sulfamic acid.

During the first 50 days, ammonium sulfate equivalent to 300 ppm N. and calcium nitrate equivalent to 150 ppm N. produced similar effect, but from 50 to 100 days the ammonium sulfate remained parallel to the control while the calcium nitrate continued depressive. Sulfamic acid produced a marked depression which was immediately evident and persisted during the first half of the incubation period. This cannot be interpreted solely on the basis of added nitrogen: subsequent studies[5] have

[5] To be published later.

shown that sulfamic acid, when applied to alkaline as well as to acid soils, is toxic to germination and growth of sunflowers, that it temporarily reduces bacterial numbers, and that it strongly depresses CO_2 evolution. The toxic effect is apparently specific since sulfite liquor, which is highly sulfonated, is nontoxic even in much greater quantities.

The depressive effect of ammonium sulfate on respiration in Willamette silty clay loam was closely proportional to the amounts applied, the equivalent of 250 ppm N. reducing CO_2 evolution about as much more as the 106 ppm N. reduced it below the control. As with Newberg loam, the nitrate was considerably more depressive than the ammonium salt.

According to Waksman (40) the decomposition of humus of only wide C: N ratio is favored by the addition of inorganic fertilizers. In line with this the wide C: N ratio of the Vale soil affords a reasonable explanation for the stimulation observed. The depressing action noted in the Newberg and Willamette soils, however, is accounted for less readily. Potter and Snyder (17) also observed that both ammonium sulfate and sodium nitrate, used in amounts equivalent in nitrogen to 106 ppm and 100 ppm respectively, decreased the quantity of CO_2 evolved from Miami silt loam, and that the nitrate was more depressive. Possible explanations of lowered respiration intensity in the presence of these nitrogen compounds are: (1) they are absorbed by the humus complex in such a way as to temporarily immobilize previously decomposable carbon compounds; (2) the added nitrogen, being directly assimilable, relieves the microbes from the necessity of decomposing organic nitrogeneous compounds for their nitrogen requirements. The latter seems more probable. Reasons why nitrates are more depressive than ammonium salts are not clear; possibly the explanation involves oxidation-reduction potentials and availability as influenced by relative diffusion rates of anions and cations. From the standpoint of availability within the cell it would seem that ammonium nitrogen would be preferable to nitrate, since the latter must be reduced before it can be synthesized into tissue.

SUMMARY AND CONCLUSIONS

Decomposition of native organic matter in ten soils, including similar and dissimilar types, was studied by the respiration method. The influence of moisture and added available nitrogen compounds on respiration in some of these soils was also investigated.

Texture, organic matter content, and C: N ratio were found to be controlling factors in CO_2 evolution.

In four soils which differed widely in texture, organic matter, and C: N ratio, the last-named exerted predominating influence on both the rate and amount of CO_2 production.

The optimum moisture content for soil respiration was found to be approximately 75 per cent of the saturation capacity. This was true in two soils of similar texture but greatly different saturation capacity. Differences in moisture produced similar respiratory response in both

soils, but the effects were quantitatively greater in the soil with greater
water capacity. It is suggested that for CO_2 production as a general
microbial function, the optimum moisture may range between rather wide
limits, whereas for strictly aerobic processes it is more sharply defined
since aeration is inversely proportional to soil moisture.

Addition of available nitrogen increased respiration in a soil con-
taining organic matter of wide C:N ratio. Depressive effects were ob-
tained in other soils; nitrates were more depressive than ammonium sul-
fate. Possible reasons for these results are discussed.

The microbial population of the various soils, which present a wide
range in origin, profile development, texture, and organic matter, was not
greatly dissimilar either at the beginning or at the close of prolonged
incubation. Outstanding respiratory differences developed during the
first few days of incubation. Had chemical and microbial analyses been
made during this time, changes correlating with CO_2 evolution would
probably have been obtained. As carried out later, however, the analyses
revealed only that different soils, as well as variously treated soils, at-
tain a similar and relatively constant microbial and chemical equilibrium.
This is likewise indicated by the similarity in slope attained by all
respiration curves as incubation proceeded.

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A PLACE FOR THE PLATE COUNT METHOD AS APPLIED TO SOIL [1]

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The plate method of estimating numbers of bacteria is almost as old as the science of bacteriology. It had its beginning in Koch's liquefiable solid medium developed in 1881. This new medium revolutionized the procedure for studying bacteria. A small amount of material under investigation was mixed with the melted gelatine medium. On cooling, the medium solidified and trapped the organisms. There, they developed to form well-isolated colonies that usually contained only one species. These could be studied as pure cultures or counted. When the procedure was carried out quantitatively the counts could be used to give an estimate of the number of bacteria per gram of the original substance. The procedure was simple and soon was applied to soil, whose population responded in large numbers to the new technique. It was accepted without seriously questioning the validity of the resulting estimates. There was not even a standardized procedure, although most investigators followed the principles laid down for counting bacteria in milk. Each laboratory had its own system of preparing dilutions and favored certain media as most suitable for the purpose intended. The method was of value only if put to practical use. Accordingly, attempts were made to apply plate counts of bacteria to problems of soil fertility and crop production. One medium and set of cultural conditions produced one estimate. Another medium or set of conditions gave one quite unlike the former. By varying these factors it was possible to obtain estimates of populations with vastly different metabolic properties. The method was used extensively. Apparently, the potential information to be derived from plate counts of bacteria from soil was almost limitless.

Herein is to be found the reason for the failure of the method to produce the results expected. It was accepted by the soil scientist interested in the solution of practical problems before it had been proven capable of producing an estimate that was reproducible within definite limits. In the early twenties of this century the accuracy of estimates produced by the plate count method was questioned, and procedures were outlined for evaluating such estimates. It became apparent that the simple procedure followed in most laboratories produced a result of doubtful value. The findings of one investigator with respect to the

relation of numbers of bacteria to a specific effect frequently were at variance with those of another. Too, there was evidence of apparent wide fluctuations in numbers of bacteria from samples taken at intervals from a given plot. This failure to confirm results, coupled with the gradual acceptance of the concept of a rapidly changing population in soil, led to an attitude of hopelessness in the minds of many workers. Consequently, counts were made and reported in a perfunctory manner along with data pertaining to biological activity and chemical and physical tests. There was little attempt to interpret counts. In short, the method was relegated to a place of minor importance in most laboratories, and other lines of approach were developed.

Large scale platings carried out at The University of Manitoba have furnished data that illustrate many reasons for the skepticism about the procedure. Composite samples from each of 36 plots were plated weekly for fourteen weeks during 1936. There was little evidence of consistency in the relation of the estimates to different fertilizer treatments or crops. To add to the confusion, estimates from 36 subsamples from one well-mixed composite of the residue from all samples were found to vary as widely as the estimates from the 36 samples from different plots. This led to a detailed consideration of the method.

THE ERRORS OF THE METHOD

The plate count procedure, as applied to plots or fields, consists of several sampling steps, each of which is subject to experimental errors. The following diagram shows the sources of variation in the method.

Since every estimate of the population in a plot is made from counts on plates and consequently is subject to errors at every step shown above, it is obvious that real differences in the bacterial population may be obscured completely by variations arising from these sources of error. Hence, it is essential that the amount of variation at each sampling step be determined, and that the sampling procedure at each step be such that differences among estimates are as small as practicable. This implies the use of a design for the experiment that provides data for measuring variation of this nature, which means at least duplicate determinations at each step.

The information available on each source of variation shown in the diagram may be considered briefly.

1. The error of replicate plates.

Every estimate of the bacterial population is made from counts on plates. Hence, the error of replicate plates is a part of every estimate, whether of a dilution, a subsample, a sample, or a plot. When duplicate or replicate determinations have been made, the mean square for replicate plates may be calculated by analysis of variance on all the data for the plot. This value may be large or small. The same data may be used to provide a X^2 (chi square) value for each set of replicate plates.

Plot 1.
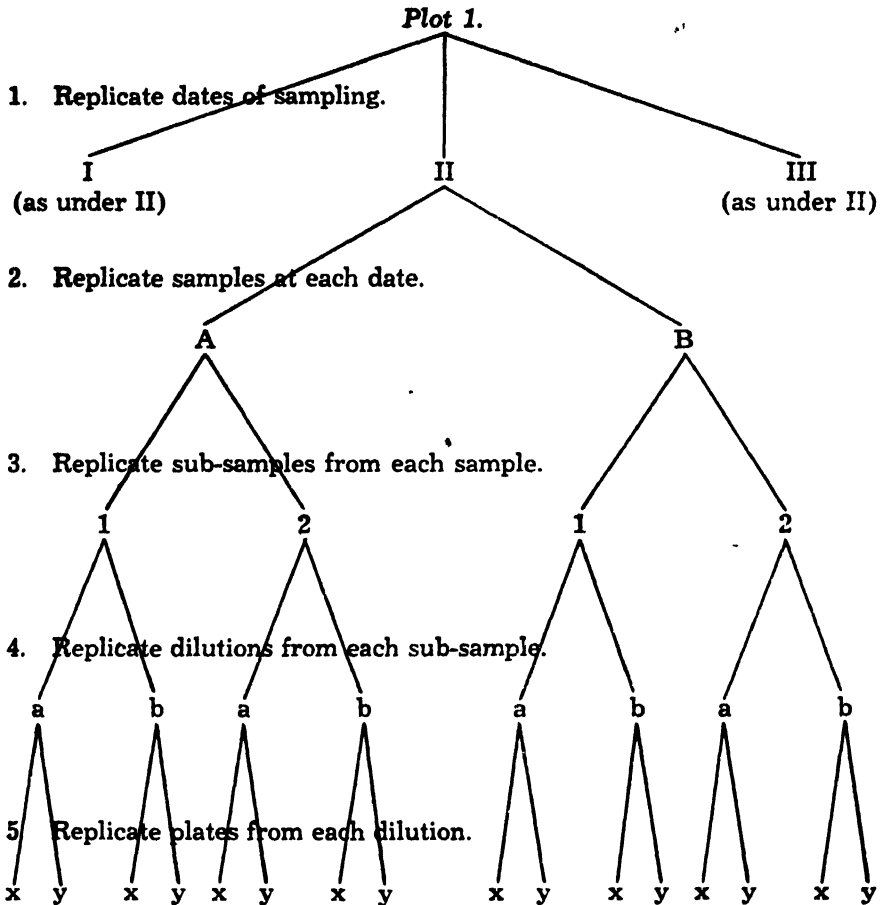
1. Replicate dates of sampling.

    I   II   III
(as under II)   (as under II)

2. Replicate samples at each date.

    A   B

3. Replicate sub-samples from each sample.

  1   2   1   2

4. Replicate dilutions from each sub-sample.

 a b a b   a b a b

5. Replicate plates from each dilution.

x y x y x y x y   x y x y x y x y

If the distribution of these X^2 values conforms to expectancy for the Poisson series, the variation among counts from replicate plates is due to random sampling and there is little hope of reducing the mean square for replicate plates by changing the procedure. A poor distribution of X^2 values, on the other hand, usually indicates the presence of a factor that causes wide variation among counts in too many sets of replicate plates. This latter condition does not appear to be associated with the technique of plating, since in this laboratory the procedure used regularly for plating produced counts that gave a X^2 distribution conforming to expectancy in an experiment in which pure cultures of bacteria were added to sterile soil just before plating. Further, the same result was obtained with the normal soil flora in samples plated within 6 hours after the samples were obtained from the experimental plots. On the contrary, the variation among counts from replicate plates from one dilution was found to be wide in too many sets of counts when samples

were broken up and held in the laboratory overnight or longer before plating. The disturbing effect increased in intensity with the time of holding. The presence of pin-point colonies of bacteria or certain species of fungi on some plates in a set likewise increased the number of sets of counts that varied widely.

The failure to randomize the order of pipetting to plates, adding medium, piling plates in the incubator, and counting might be expected to produce a large error of replicate plates. This has been found not to be the case in spite of the fact that these procedures have been carried out deliberately in one order in all our experiments. Accordingly, the effect of the systematic order of the procedure may be disregarded; and the differences among replicate plates from one dilution accepted as the minimum laboratory error, against which the significance of differences among replicate dilutions from one subsample may be tested.

2. Differences among replicate dilutions.

An estimate of the population in a dilution is based on counts from plates. Accordingly, the mean square for replicate dilutions in general will not be less, and may be several times as large as the mean square for replicate plates. The success of the diluting procedure in keeping the differences among dilutions at a minimum depends largely on maintaining a uniform suspension of the soil in the water of the initial dilution blank at the time repeated transfers are made to the next higher dilution blanks. At the beginning of our studies the usual procedure was to suspend 25 grams of soil in 250 ml. of sterile water and shake mechanically on a to-and-fro shaker for 5 minutes. This gave a mean square for dilutions about three times that for replicate plates. This weakness in the procedure has been overcome under our conditions of experimentation by using a suspension of 50 grams of soil in 1,250 ml. of water, shaking for 10 minutes on a mechanical shaker and agitating as repeated transfers are made to the series of final dilutions for plating. Under this condition the mean square for dilutions is only slightly larger than that for replicate plates in two experiments completed recently.

The range in the number of colonies allowable on plates is worthy of consideration, since it is conceivable that an estimate based on a dilution yielding a count of 20 colonies per plate may not agree with another from the same sample based on a dilution giving 200 colonies per plate. This discrepancy may be associated with the wide differences in the number of colonies in plates with the same area and amount of food material. The effect may be attributable to: (a) failure of some colonies to grow on the crowded plate because of lack of available nutrients, (b) failure to grow because of the presence of toxic products of metabolism produced by certain members of the population, or (c) stimulation of some colonies by products and possibly growth stimulants produced by other kinds of bacteria.

Obviously, the only method of regulating the number of colonies on plates is by changing the strength of the dilution plated. This effect has

been tested in our laboratory. Each subsample was diluted in three different strengths calculated to give theoretical counts in the ratio of 3:5:8. Counts from pairs of dilutions were correlated. It was readily apparent that the dilutions did not give proportional counts. The high count gave an estimate relatively lower than the low count from a dilution of the same sample; and this discrepancy increased with the size of the counts. At the same time the mean square for dilutions on the basis of the number per gram of dry soil for each dilution was about twice that for replicate plates. However, the correlation between pairs of dilutions was so strong that counts from one dilution could be adjusted to make the estimate interchangeable with that based on counts from another dilution of different strength. The mean square for dilutions on the basis of adjusted data was only slightly larger than that for replicate plates. These findings make it obvious that the effect associated with different numbers of colonies on plates varies in a regular manner; and that the design of an experiment should provide a means for minimizing this effect. Accordingly, the use of three dilutions of different strengths gives three chances of having the most suitable dilution at each date of sampling from one source in an experiment extending over a crop season. Too, it furnishes a means of compensating for differences in the number of colonies per plate.

3. Differences among replicate subsamples.

Similarly, an estimate of the population in a subsample is based on counts from plates made from dilutions. It is subject to the effects of differences among plates and among dilutions, and in addition to the variation arising from the process of subsampling. The success of the subsampling process in furnishing a representative portion of the sample depends upon the ease of reducing the sample to uniformly small particles and the efficiency of mixing. These are functions of the texture of the soil and its moisture content. In the first studies on this project in our laboratory, when the samples of soil were broken and mixed by hand, the mean square for subsamples was many times that for replicate plates. This variation among subsamples has been reduced appreciably under our conditions by grating the soil mechanically through a four-mesh-per-inch wire screen under slight pressure and mixing in a revolving drum for 10 minutes. While the mean square for subsamples was larger than that for replicate plates in samples handled in this way, it is possible that this variation has been reduced as much as is practicable.

4. Differences among replicate samples.

An estimate of the population in a sample is subject to the effects of differences introduced by all the preceding sampling steps; as well as to the difficulty of obtaining samples with the same population levels from the plot. The variation among replicate samples is a function of soil heterogeneity in the area sampled. There is little hope of changing this variability. One may assume that under certain conditions this discrepancy may be overcome by using composite samples consisting of larger

numbers of cores taken from points determined at random from the plot. This assumption has not been substantiated under our conditions. On the contrary, the variation in estimates among samples consisting of 24 cores was about the same as that among samples composed of 6 cores. The same was true for composite samples of 18 and 12 cores. This might be interpreted as indicating that the variability within a core 6 inches long and 1 inch in diameter was as great as that among cores. Under this circumstance there could be little chance of reducing differences among samples by using a larger number of cores per sample. Further, the problem of soil heterogeneity in a plot or field may be linked with that of variability in moisture content. A strong correlation between moisture expressed in percentage and bacteria expressed in millions per gram is known to exist. Since in our experiments the variation in moisture among samples taken from a plot at one time has been shown to be wide enough to cause marked differences in estimates, it is obvious that a portion of the differences among samples may be accounted for by differences in their moisture content. This points to the need for a moisture determination on every sample plated; and also for considering the effect of moisture in the interpretation of an estimate of the bacterial population.

5. Differences among replicate dates of sampling.

Obviously, differences among estimates of the population in a plot made on different dates result from all the sources of variation already considered; and also from the effects of day-to-day or seasonal variation. These changes in numbers of bacteria may be associated with differences in one or many environmental factors. In our laboratory, changes in the moisture content of the plot have been found to account for a large portion of the mean square for differences among dates of sampling. In some plots an effect associated with the kind of crop and the stage of its development has been shown to be responsible for another equally large portion. Still, other effects may be found to account for other portions of the mean squares for differences among dates. A few of these follow: differences in the temperature of the soil on the dates of sampling, differences in length of time since the temperature was at its maximum, differences in the length of time after a rain, and differences in the amount of rainfall. It is apparent that the fluctuations in estimates of the population of a plot result from the combined response to changes in a number of factors. The portion of the mean square for differences among dates attributable to each factor must be known; and the possible effect of other disturbances must be realized. Then only, the residual or remaining portion of the mean square for differences among dates may be interpreted in relation to the specific effect under consideration.

PRINCIPLES AND NOT PROCEDURES

The principles governing the revision of the method for estimating numbers of bacteria are more important than the details of the laboratory procedure. The method as applied to soil deals with biological populations

varying at random and living in a medium that lacks homogeneity. The procedure consists of a series of steps involving random sampling and, as well, additional variation at each step. The practice of replication makes it possible to consider the amount of disturbance at each source of error as it affects the experiment as a whole. Revisions of the method must be considered with respect to the precision of the final estimate. Certain refinements regarding the tolerance of dilution blanks and pipettes may produce theoretical improvements that may be within the limits of experimental error for the method. Another refinement in the procedure, such as using a larger proportion of water to soil in the original dilution, may yield a significantly better estimate. This means that revisions must be rated in terms of relative importance and with due consideration to practicability. Further, the use of replicates gives a final estimate based on an average of two or more random samples at each step in the procedure. The number of replications will be determined by the precision desired in relation to the amount of labor and materials involved in obtaining the estimate. The last general principle to be considered involves the interpretation of the result. Estimates have been found to be subject to sampling variation at many places in the procedure. Too, the population has been shown to be responsive to various environmental factors. The problem of whether the difference in estimates represents response to a specific effect under investigation, or merely is the result of sampling variation or a combination of other effects or both, must be settled by a method of handling data that provides a mathematical value for each effect. Statistical methods have been developed for treating such data and for determining whether the variation among estimates represents only sampling variation or, in addition, response to some other factor. This method of analysis depends upon replication at each sampling step. It provides a constant check on known effects and a means for testing new ones. Only after such consideration can an estimate of the bacterial population in soil have real value.

A PLACE FOR THE METHOD

In spite of the recognized limitations of the plate count method, a series of estimates from a plot obtained by a procedure based on the principles outlined will have value in establishing the relationship of bacteria in soil to problems of importance to agriculture. This can be stated with confidence because of three facts.

1. Replication of samples at each step involving random sampling from a biological population results in a better estimate than one based on counts from one dilution only.

2. This replication provides a means of measuring the variation at each step in the procedure. Each source of discrepancy may be considered in true perspective with regard to the final estimate and the technique improved where serious disturbances are found.

3. The method is extremely sensitive in demonstrating responses

to changes in moisture and cropping. It is probable that it is equally responsive to other environmental factors.

These facts may be interpreted in relation to the inadequacy of the method in the past. They may be used to explain many of the inconsistencies that have been charged against the method. In the light of present knowledge, it is conceivable that an estimate could be so affected by failure to provide for the difficulties of sampling, or to consider response to environmental factors, that a real response to an effect under investigation could not be detected.

The same facts may be considered with respect to the use of the method in the future. In an experiment carried on recently under the conditions at The University of Manitoba, 60 to 90 per cent of the mean squares for the so-called seasonal fluctuations in the bacterial population were found to be associated with response to changes in moisture and in the development of crops. This extreme sensitivity to environmental conditions should make the method valuable for investigating the relationship of the microflora of the soil to plant growth in experimental plots. This type of study has many applications with respect to such problems as crop rotations, the use of fertilizers, and the control of soil-borne plant disease organisms. Too, it may be used to demonstrate responses by widely differing populations in the soil. Now that the soundness of the procedure is established, more emphasis may be placed on the use of specific media for various populations. In this respect it is highly probable that a carefully chosen selective medium may show that the population developing on it responds more markedly to a specific effect than does the general population to be found on a nonselective medium. This opens the road to a vast field, as yet unexplored. It involves the use of media containing products of the decomposition of plant materials and of root secretions.

When estimates based on single samples are considered, the marked response to moisture and cropping becomes a large and serious error. Since there is no means of estimating and removing the effects of these population responses in single estimates from plots, the sensitivity of the experiment is reduced sharply. This is because the response to these factors increases the only error available for testing the significance of the effect considered. A biological response to differences in chemical composition of the soil could not be detected unless it was several times as large as the combined variation resulting from random sampling at each step and the responses to moisture and cropping. Any smaller, even though real, population response would be masked completely by the large error of the experiment. The same reasoning applies to single samples from widely separated areas representing different soil types. The error in an experiment of this kind is likely to be so large that only an extreme response to an effect could be detected.

The sensitivity of the method in demonstrating response of the population to certain environmental factors, even with a nonselective medium,

gives reason for hope that it may be adapted to detect deficiency in mineral elements in soil with a precision not equalled by any chemical or biological method used for that purpose today. It is conceivable that a medium may be developed for growing a population so highly specialized in growth requirements as to respond to differences in amounts of certain of these elements that are within the limits of experimental error of recognized procedures.

Whether the method based on the principles outlined is used in intensive experimental plot studies or whether it is adapted along some of the lines indicated, undoubtedly it will find application in the problems so aptly referred to by the late Dr. Brown as he neared the end of a life devoted to this "fascinating and important science," when he expressed his views as follows[2]: ". . . it is particularly important that the true character of the soil and the occurrence and activities of micro-organisms in it should be recognized and appreciated. . . . Much remains to be learned of the processes in the soil, of the relationships between various micro-organisms, of the dependence of the organisms upon soil conditions and of the relations of the different processes to crop growth, to soil conservation, and to permanent agriculture."

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[2] Soil Science 40: 49–58, 1935.

INFLUENCE OF THE DECOMPOSITION OF ORGANIC MATERIALS ON SOME PROPERTIES OF ALKALINE-CALCAREOUS SOILS

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The influence of decomposing organic materials on the physical, chemical, and microbiological properties of soils has received the attention of many investigators in recent years (1, 3, 4, 6, 10, 12, 13, 17, 18, 19, 20, 23, 26, 27, 28, 29, 30, 31, 38). Among the benefits of organic matter additions to soil are: improved structure, increased water-holding and base-exchange capacities, decreased susceptibility to erosion, increased availability of nutrients such as phosphorus, stimulated production of "auximones," decreased pH values and more rapid reclamation of alkali soils, and increased activity of the micropopulation.

Dr. P. E. Brown and his colleagues, in a series of papers dating from 1915, contributed much of the pioneer work in this field of study (7, 9, 24, 25, 33, 34). Doctor Brown emphasized particularly the importance in the growth of crops of available nitrogen (7), and revealed the relationship between decomposing organic matter and groups of soil micro-organisms physiologically active in nitrogen transformations (9). He and his colleagues established a relationship between the nitrogen present in organic materials and their rate of decomposition (25), and the influence of such material on the nitrogen content of the soil (24). These workers showed further that the base-exchange capacity of the soil can be increased by organic matter additions (24), and that the decomposition of these materials in the soil markedly increases the infiltration rates (34).

In the alkaline (pH 7.3-9.4), calcareous ($CaCO_3$, 2-20 per cent) soils of the Southwest, the decomposition of organic matter creates problems peculiar to the region. Since the annual precipitation is scarcely more than 10 inches, irrigation is necessary for crop production. Temperatures average approximately 80° F. from May to September, and 55° F. the balance of the year. These environmental conditions cause a rapid, comparatively complete decomposition of any added organic materials with the result that little accumulation occurs, and the organic matter content of the soil seldom exceeds 1 per cent.

Because of the rapid decomposition, and consequent slight accumulation of organic residues in the Southwest, many farmers question the economy of organic matter additions. It was deemed advisable, therefore, to initiate a study of the rates of decomposition of typical plant materials in this region to correct this misconception, and in addition, to study the influence of the decomposition process on the soils' pH value, nitrate-

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nitrogen content, and structure under laboratory conditions. The information thus obtained may serve as a foundation upon which to establish a program for improving the crop-producing power of the soils of this area. Such preliminary studies are reported in the present investigation.

EXPERIMENTAL

METHODS AND PROCEDURE

Three soils typical of scattered agricultural regions of Arizona were selected: (a) *Pima clay loam* from Safford (taken from a field fertilized annually for the past ten years with manure at the rate of 12 tons per acre); (b) *Laveen sandy loam* from the State Agricultural Experiment Farm at Mesa; and (c) *Superstition sand* from the Yuma mesa. *Clarion silt loam*,[2] representative of soils of more humid regions, was used for comparison. Descriptions of each soil type may be obtained from the appropriate Soil Survey reports (8, 35). The soils, freshly collected from the field, were screened through a 10-mesh sieve and thoroughly mixed to insure uniform sampling.

Two leguminous and two nonleguminous plant materials, sesbania and sour clover, and hegari and Markton oats, respectively, were chosen for the experiment. These plant materials are typical of those commonly added in large amounts to Arizona soils. The plants were gathered just prior to maturity, dried in the shade, and ground to pass a 20-mesh sieve. Each of the plant materials was incorporated with 150 grams of soil in glass tumblers at the rate of 2 per cent and was uniformly mixed. Enough distilled water was added to each sample to bring moisture up to optimum (approximately 65 per cent of the water-holding capacity). Untreated samples of each soil were incubated also. The tumblers, fitted with loose covers, were placed in a thermostatically-controlled incubator at 30° C. From time to time, enough water was added to maintain the samples at optimum moisture content.

Duplicate samples of each soil treatment were removed periodically from the incubator, the soil in each thoroughly mixed, and aliquots taken for determination of the pH value, nitrate-nitrogen and total carbon contents, and degree of particle aggregation.

Fifty-gram samples (on a dry-weight basis) of the mixed soil were transferred to wide-mouth bottles; 250 ml. of distilled water were added, and the bottles were shaken for 20 minutes on a mechanical shaker. Determinations of pH were made on this 1:5 soil-water suspension with a Beckman pH meter. The suspension was then filtered. Nitrates were determined in the filtrate by the phenoldisulfonic acid method (2). When necessary, the solutions were clarified and decolorized by Harper's cupric sulfate-calcium hydroxide method (16). The color comparisons were made with a Cenco photelometer.

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[2] Obtained through the courtesy of Prof. B. J. Firkins, Department of Agronomy, Iowa State College, Ames, Iowa.

One-to-three grams of each soil were used for the total carbon determinations, depending on the carbon content; analyses were made by the Walkley-Black modification (40) of Schollenberger's dichromate titration method (32).

The effect of the organic materials on soil structure was deduced from the degree of aggregation of the soil particles. An adaptation, proposed by Gerdel (15), of the Bouyoucos hydrometer method of mechani-

TABLE 1

CHANGES IN CERTAIN PROPERTIES OF PIMA CLAY LOAM WITH TIME DURING THE DECOMPOSITION OF ORGANIC MATERIALS

Treatment	Incubation Time in Days	Total Carbon Content (%)	pH	Nitrate-N Content (ppm)	Particles Less Than 0.05 mm. (%)
No treatment	0	1.24	8 46	52.0	42
	3	1.25	8.45	50.4	42
	7	1.25	8.45	58.4	39
	15	1.26	8.47	55.8	38
	28	1.24	8.42	90.5	38
	49	1.22	8.38	94.8	38
	70	1.23	8.40	116.0	39
	133	1.24	8.35	148.5	37
Sesbania (2%)	0	2.12	7.88	52.0	42
	3	1.91	8.05	3.2	41
	7	1.82	8.18	26.6	36
	15	1.72	8.11	214.1	33
	28	1.67	8.01	339.5	32
	49	1.64	7.92	378.0	31
	70	1.57	7.90	412.0	30
	133	1.57	7.92	565.0	29
Sour clover (2%)	0	2.02	7.85	52.0	40
	3	1.82	8.08	20.0	38
	7	1.68	8.31	58.7	34
	15	1.60	8.14	274.6	34
	28	1.58	8.02	393.0	33
	49	1.56	7.95	442.0	31
	70	1.48	7.97	460.0	32
	133	1.45	7.91	594.0	30
Hegari (2%)	0	2.14	7.95	52.0	40
	3	1.95	8.00	1.5	36
	7	1.87	8.16	0.6	34
	15	1.76	8.52	0.7	32
	28	1.64	8.49	1.8	30
	49	1.62	8.36	27.7	30
	70	1.54	8.43	54.7	30
	133	1.51	8.22	151.5	26
Markton oats (2%)	0	2.18	8.06	52.0	40
	3	2.05	8.09	1.4	34
	7	1.96	8.33	0.6	33
	15	1.87	8.60	0.5	27
	28	1.70	8.58	0.9	28
	49	1.63	8.48	7.4	30
	70	1.49	8.43	73.9	29
	133	1.48	8.31	122.0	24

TABLE 2

CHANGES IN CERTAIN PROPERTIES OF CLARION SILT LOAM WITH TIME DURING THE DECOMPOSITION OF ORGANIC MATERIALS

Treatment	Incubation Time in Days	Total Carbon Content (%)	pH	Nitrate-N Content (ppm)	Particles Less Than 0.05 mm. (%)
No treatment	0	2.02	5.40	60.0	38
	3	1.98	5.29	58.2	36
	7	1.86	5.30	58.9	34
	15	1.84	5.25	61.5	34
	29	1.89	5.22	91.5	35
	50	1.87	5.19	107.5	32
	71	1.87	5.04	105.5	32
	133	1.79	4.92	161.0	28
Sesbania (2%)	0	2.88	5.34	60.0	39
	3	2.60	6.34	4.1	37
	7	2.52	7.00	0.9	34
	15	2.41	7.23	9.1	32
	29	2.36	7.07	13.7	27
	50	2.33	5.96	164.5	24
	71	2.21	5.21	300.0	21
	133	2.18	4.58	509.0	19
Sour clover (2%)	0	2.79	5.39	60.0	40
	3	2.65	6.18	46.9	39
	7	2.47	6.85	16.8	33
	15	2.32	7.48	6.8	32
	29	2.26	7.49	8.0	30
	50	2.18	6.75	54.5	26
	71	2.05	6.05	218.0	26
	133	2.06	5.19	440.0	26
Hegari (2%)	0	2.92	5.35	60.0	38
	3	2.66	5.75	6.2	36
	7	2.54	6.71	0.7	32
	15	2.42	6.14	0.5	26
	29	2.30	5.99	0.9	23
	50	2.26	5.87	9.1	21
	71	2.15	5.34	71.4	19
	133	2.05	5.02	163.0	14
Markton oats (2%)	0	2.93	5.40	60.0	38
	3	2.62	5.86	3.7	35
	7	2.46	6.63	0.2	30
	15	2.39	6.12	0.6	22
	29	2.34	6.01	4.3	20
	50	2.21	5.53	60.5	18
	71	2.21	5.21	300.0	21
	133	2.04	4.94	187.5	15

cal analysis (5) was used to determine the degree of aggregation. The soil was put into Bouyoucos cylinders with 1,000 ml. of distilled water and let stand overnight. The following morning the cylinders were turned end-over-end 20 times by hand, and promptly replaced on the laboratory desk. The percentage of particles less than 0.05 mm. in diameter was determined by taking a hydrometer reading after the suspension had been allowed to stand exactly 40 seconds. Proper temperature corrections were applied to all hydrometer readings.

A comparison of the percentages of particles less than 0.05 mm. in diameter in the untreated and in the treated samples reveals the influence of organic matter decomposition on the water-stable aggregates of the soil. It was necessary to bring the soil samples to optimum moisture 24 hours prior to analysis to obtain consistent results by this method.

<div align="center">RESULTS</div>

Notwithstanding a wide range of values for the physical and chemical properties of the three alkaline, calcareous soils used (texture, sand to clay loam; total carbon, 0.04-1.24 per cent; total soluble salts, 300-2,000 ppm; nitrate-nitrogen, 2.5-52 ppm; pH value, 8.46-8.79), nearly identical changes, differing only in magnitude, were observed in the three soils during decomposition of the organic materials.

For the sake of brevity, the results for Pima clay loam only are presented here, and these data together with those obtained from Clarion silt loam are given in Tables 1 and 2.

<div align="center">RATE OF DECOMPOSITION</div>

The decrease in carbon content in the variously treated Pima clay loam samples is presented in Figure 1. These curves, typical of those obtained with the other soil samples, show that the rate of decomposition of the plant substances was most rapid during the first two weeks of decomposition. Complete decomposition had not yet occurred at the end of 133 days of incubation, the duration of the experiment.

The data in Tables 1 and 2 and Figure 1 indicate that the various materials decompose at different rates in any given soil, and that the rates vary from soil to soil. The extent of decomposition, in percentage of the carbon originally added, is shown for various incubation periods in Table 3. The 15-day period was chosen for comparison because the rates of decomposition prior to and following the fifteenth day of incubation are quite different; thus the data indicate the influence of the initial rate on the extent of decomposition observed at the end of 133 days.

Initially the decomposition rate was more rapid in the Pima clay loam for the leguminous than for the nonleguminous plant materials, whereas the rates were about equal in the Clarion silt loam. After 133 days of incubation, however, a greater percentage of the nonleguminous carbon had been oxidized than of the leguminous. Sour clover decomposed more rapidly than sesbania and almost as quickly as the oats and hegari; the latter two substances decomposed at approximately the same speed.

Chemical analyses of the plant materials showed 45 per cent of the sour clover to be water soluble, whereas only 25 per cent of the sesbania was soluble. That the sour clover should decompose more rapidly than the sesbania is evident, therefore, since Waksman and Tenney (39) and others have shown that the water-soluble fraction of organic materials decomposes most rapidly.
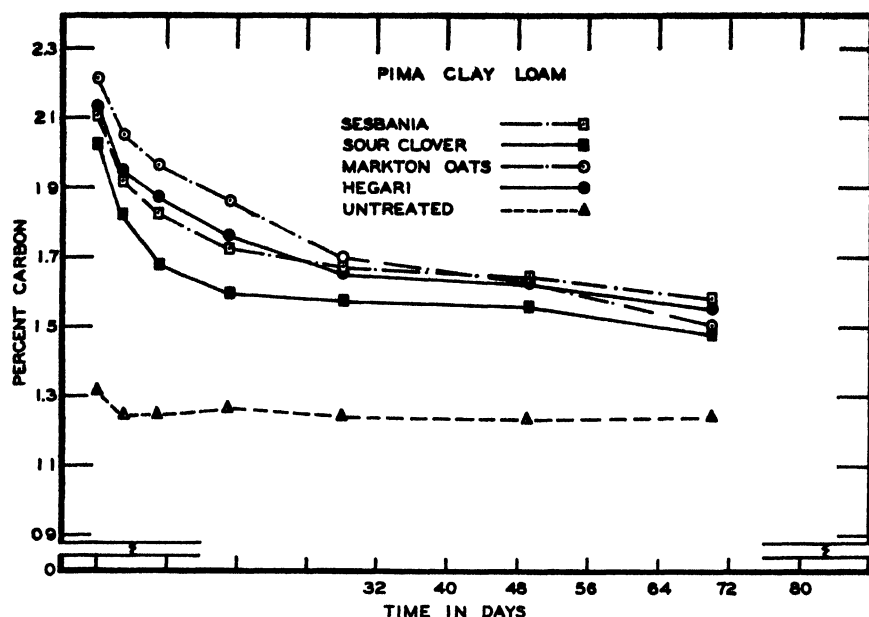
FIG. 1. Changes in carbon contents of samples of Pima clay loam during the decomposition of organic materials.

The rapid rate of decomposition of the nonleguminous materials may be explained as follows: Since a smaller percentage of nitrogen is present in the oats and hegari (1.35 per cent) than in the clover and sesbania (3.5 per cent), less protoplasm can be synthesized at any given time by the heterotrophes attacking the organic materials; consequently,

TABLE 3

TOTAL CARBON LOSS WITH TIME IN PIMA CLAY LOAM AND CLARION SILT LOAM SOILS
VARIOUSLY TREATED WITH ORGANIC MATERIALS

Treatment	Incubation Time in Days	PIMA CLAY LOAM		CLARION SILT LOAM	
		Gms C per 100 Gms. Soil	Loss in Weight (%)	Gms. C per 100 Gms. Soil	Loss in Weight (%)
Sesbania	0	0.85	0	0.86	0
	15	0.48	45.5	0.39	54.6
	133	0.33	62.5	0.16	81.4
Sour clover	0	0.78	0	0.77	0
	15	0.36	53.9	0.30	61.0
	133	0.21	73.1	0.04	94.9
Hegari	0	0.90	0	0.90	0
	15	0.52	42.2	0.40	55.6
	133	0.27	70.0	0.03	96.5
Markton oats	0	0.94	0	0.91	0
	15	0.63	33.0	0.37	59.4
	133	0.24	74.5	0.02	98.0

over a long period of time a larger carbon loss would result from the nonleguminous than from the leguminous substances.

The fact that the organic additions decomposed more rapidly in the *acid* Clarion silt loam than in the *alkaline* Pima clay loam is of particular interest. In general the assumption has been made that organic matter decomposes more quickly in alkaline than in acid soils by virtue of factors other than the higher prevailing temperatures in arid regions (28). The data presented herein, however, indicate that such is not the case since identical experimental conditions were used in the comparison of the two types of soils. Stephenson (36), too, found the decomposition of organic materials to be more rapid in acid than in alkaline soils.

If the molds are more numerous in acid than in alkaline soils, as is commonly believed, and are more active organic-matter decomposers than the other soil micro-organisms, an explanation for the above findings is apparent.

pH EFFECTS

Changes in the pH values of the two soils concurrent with decomposition of the organic materials may be noted in Tables 1 and 2, and in Figures 2 and 3.

The organic matter additions had a marked effect on the pH values not only initially but during the entire course of decomposition. In the Pima clay loam, an average initial lowering of 0.5 of a pH unit occurred; the sesbania and sour clover caused an average lowering of 0.6 of a pH unit compared with 0.4 of a pH unit for the oats and hegari.

Following the initial depression in the alkaline soil, the pH values of all treated samples rose quickly, and in those treated with oats and hegari assumed a position slightly above that of the untreated samples, remaining constant thereafter or decreasing slightly to that of the untreated samples. In the case of the clover- and sesbania-treated samples, however, the rise in pH value following the initial decrease was interrupted at the end of seven days and again started to decrease. Following the twenty-eighth day of incubation, the pH values of these samples remained nearly constant at a value approximately 0.4 of a pH unit below that of the untreated samples.

In contrast to the effects noted for the Pima clay loam, the pH value of the Clarion silt loam was not appreciably affected initially by any of the organic substances. Their decomposition during incubation, however, very markedly affected the pH value. Within three days, the pH had risen about 0.5 of a pH unit in the case of the hegari- and oat-treated samples, and nearly 1 pH unit in the leguminous-treated samples. The maximum pH rise for the former was from pH 5.35 to pH 6.70, and was observed after seven days of incubation. After this maximum had been reached, the pH values decreased gradually throughout the incubation period to a value nearly the same as that of the untreated samples,
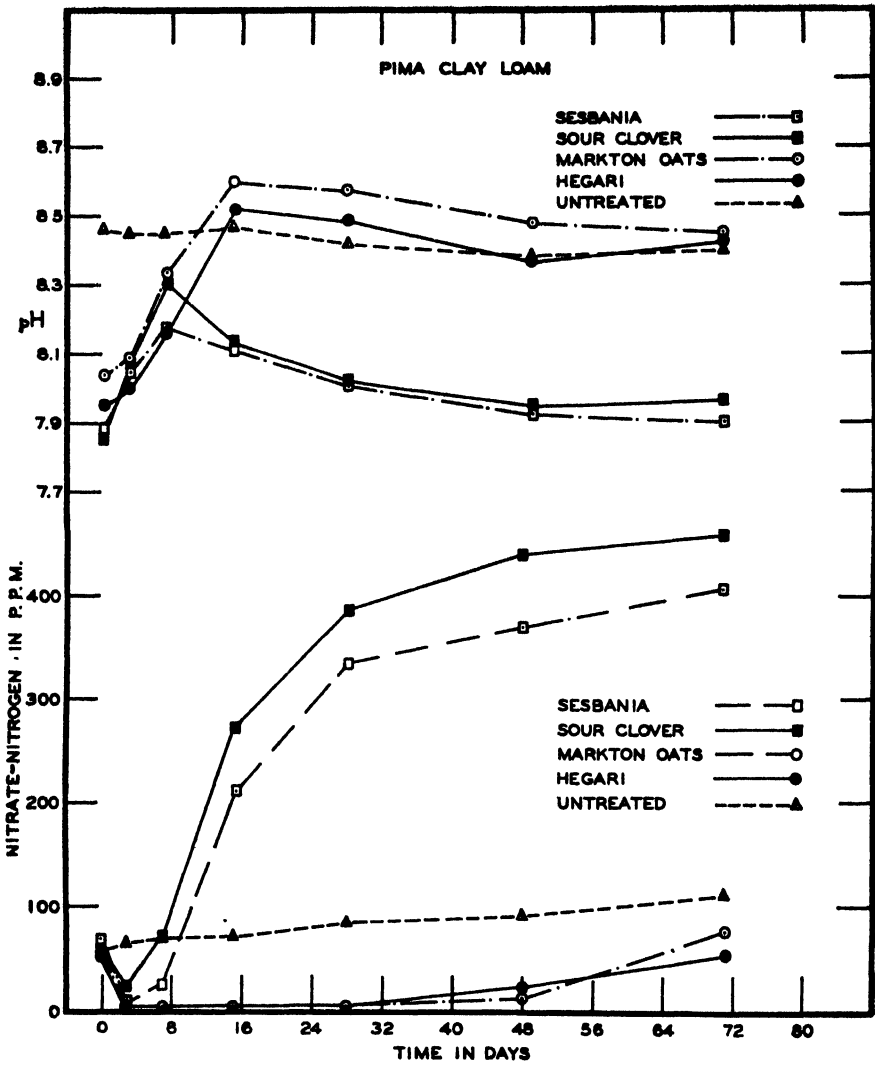
FIG. 2. Changes in pH values and in nitrate-nitrogen contents of samples of Pima clay
loam during the decomposition of organic materials.

namely, pH 4.92. The maximum pH rise for the sesbania- and sour
clover-treated samples, on the other hand, did not occur until after 15
days of incubation; the highest value reached by the sesbania-treated
samples was pH 7.23, that by the sour clover-treated samples, pH 7.49.
These values represent an average increase of over 2 pH units for the
legume-treated samples. After reaching the maxima, these curves also
show decreases in pH values, with the sesbania-treated samples decreas-
ing more quickly than those treated with sour clover. At the end of the
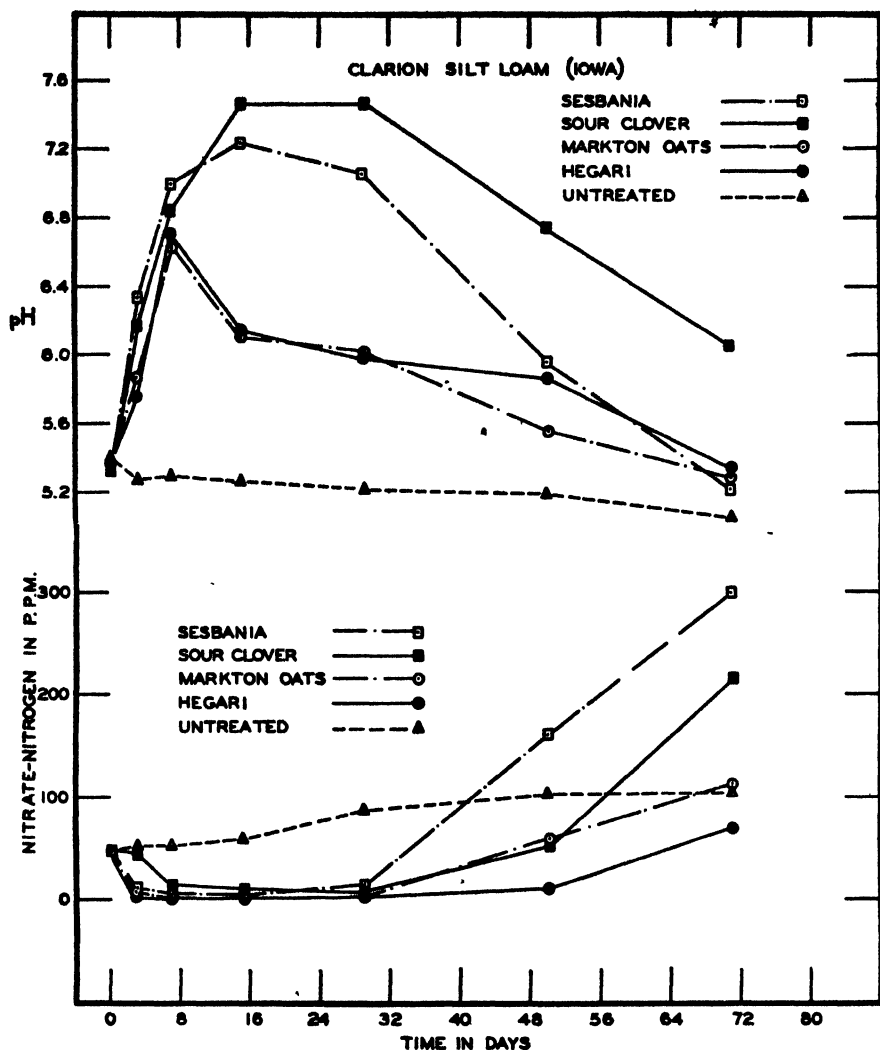
FIG. 3. Changes in pH values and in nitrate-nitrogen contents of samples of Clarion silt loam during the decomposition of organic materials.

incubation period, the sesbania-treated samples had a pH value 0.34 of a pH unit *lower,* and the sour clover-treated samples a value 0.27 of a unit *higher* than the control samples.

The foregoing pH effects are very striking, particularly in view of the observations of Oberholzer (28) that organic matter decomposition does not produce appreciable changes in the pH value of alkaline-calcareous soils. They are in agreement, however, with the findings of Conrad (13), and those of Dyal *et al.* (14).

The changes in pH reported here may be related to the following fundamental factors: (a) the production of considerable quantities of carbon dioxide during incubation, (b) the release of ammonia and its subsequent oxidation to nitric acid, (c) the inherent acidity of the water extracts of organic material, and (d) the high calcium content of the leguminous plant materials (approximately 1 per cent) which tends to increase the alkalinity of the soil as it is released during decomposition.

It is true, of course, that before any marked change in pH value can occur, the buffer capacity of the soil must be overcome. A suspension of 2 grams of the nonleguminous plant materials in 500 ml. of distilled water had an average pH value of 6.5; the corresponding value for oats and hegari was 6.25. The addition of 2 grams of these materials to the Pima clay loam samples probably explains the initial drop in pH with this soil when the values were determined on the 1:5 suspension. The absence of a corresponding increase in the pH value when these materials were added to the acid Clarion silt loam may indicate buffer action.

The changes in pH values subsequent to those occurring initially undoubtedly resulted from the products of the decomposition of the organic materials. Of these products, ammonia and its subsequent oxidation to nitric acid is probably the most effective.

NITRATE-NITROGEN CHANGES

Changes in the nitrate-nitrogen contents of the two soils during decomposition of the organic materials is depicted in Figures 2 and 3 as well as changes in pH. This dual presentation on each figure facilitates comparisons.

The rapid microbial decomposition of the added carbonaceous materials caused an initial decrease in the nitrate-nitrogen content of both soils. The low C:N ratio in the leguminous materials (15.8 in sesbania and 14.0 in sour clover), the rapid dissolution of the carbonaceous materials, and the high rate of nitrification of the released ammonia resulted in nitrates beginning to accumulate in the incredibly short time of one week of incubation. After two weeks of incubation nitrates were present to the extent of over 200 ppm, and after 133 days, over 550 ppm, in the legume-treated samples. Sour clover decomposed more rapidly than the sesbania, had the narrower C:N ratio, and showed the most rapid accumulation of nitrates.

The leguminous-treated samples of Clarion silt loam differed markedly in nitrification behavior from the correspondingly treated samples of Pima clay loam. Nitrate accumulations did not begin until the twenty-eighth day of incubation, and then at such a slow rate that an additional 12 days elapsed before the nitrate content of the sesbania-treated samples equalled that of the untreated samples and an additional 28 days in the case of the sour clover samples. When nitrates first appeared in the Clarion silt loam on the twenty-eighth day of incubation, over 60 per cent of the nitrogen in the corresponding samples of Pima

clay loam had already been oxidized to nitrates. Since dissipation of carbon was most rapid in the Clarion silt loam (Table 3), these results seem to indicate that the nitrifying bacteria are much more active in the alkaline Arizona soil.

In spite of the fact that sour clover contained the higher nitrogen content and decomposed more rapidly than sesbania in the Clarion silt loam, nitrates accumulated less rapidly in this soil treated with sour clover than with sesbania. No immediate explanation is offered for this unexpected observation.

The nonleguminous materials affected the nitrates in a similar manner in both soils. As anticipated, nitrates disappeared immediately and did not reappear for nearly seven weeks. The nitrate-nitrogen contents of the oat- and hegari-treated samples did not equal or approach that of the untreated samples until after ten weeks of incubation. In all instances, nitrates accumulated slightly more rapidly in the samples treated with Markton oats than in those treated with hegari.

CORRELATION BETWEEN NITRATE FORMATION AND CHANGES IN pH

A correlation exists between the rate of accumulation of nitrates in the soil samples treated with the leguminous plant materials, and changes in the pH values of the soils, as shown in Figures 2 and 3. In the Pima clay loam, for example, the sesbania- and sour clover-treated samples showed a distinct reversal in the pH trend when nitrates first began to accumulate after 7 days of incubation; thereafter the pH values decreased with increasing accumulation of nitric acid. The correlation is even more striking in the Clarion silt loam. A phenomenal increase of nearly 2 pH units occurred in the leguminous-treated samples during the first two weeks of incubation, and decreasing pH values were not noted until nitrates began to accumulate. A positive correlation is observed between the *rates* of nitrification and *rates* of decrease in pH also; more rapid nitrate accumulation and more rapid decrease in pH were noted with sesbania- than with the sour clover-treated samples.

An explanation for these phenomena is found in the following facts. Large amounts of nitrogen have been added to the soil in the leguminous plant material. This nitrogen, although present in complex nitrogenous or proteinaceous forms, is quickly released as ammonia by the ammonifying heterotrophes. The ammonium ion thus formed replaced sodium from the base-exchange complex of the Pima clay loam. The replaced sodium ion then combines with the carbonic acid resulting from the decomposition of the organic materials to form sodium carbonate. Hydrolysis of the sodium carbonate produces sodium hydroxide which imparts an alkaline reaction to the soil.

The exchange complex of the Clarion silt loam, on the other hand, is probably hydrogen saturated, so that the ammonium hydroxide reacts directly with the hydrogen clay to produce ammonium clay and water.

Since each of the leguminous materials contained approximately

1 per cent calcium, it is probable that large amounts of calcium, in addition to ammonia, were released during decomposition. Calcium ion thus released would tend to increase the basicity of the soil solution in the same manner that it does when added to an acid soil as lime.

Because of the heterotrophic nature of the majority of soil microorganisms, the ammonification process is rapid in both acid and alkaline soils. The oxidation of the ammonia thus formed to nitrous and nitric acids imparts an acid pH to the soil in direct contrast to the alkalizing action of the ammonia and calcium carbonate. It follows, then, that the relative rate of nitrification (or ammonia removal) determines whether the legume-treated samples become highly alkaline or acid—the magnitude of the effect depending, of course, on the buffer capacity of the soil.

The respective optimum pH values for the growth and activity of the nitritifiers and nitratifiers (37) are 7.8 and 7.1; therefore, the oxidation of the ammonia to nitrites and nitrates should be more rapid in alkaline and less rapid in acid soils. The surprisingly rapid formation of nitrates in the alkaline Pima clay loam in contrast to the sluggish formation in the acid Clarion silt loam has already been noted. It is believed, therefore, that the extremely high pH of the legume-treated Clarion silt loam samples prior to nitrate formation may be due to the accumulation of ammonia, and possibly, in part, due to the formation of calcium carbonate. The fact that the legume-treated samples of Pima clay loam never became as basic as the untreated samples probably results from the extremely rapid rate at which nitrates accumulated, thus rendering the soil less alkaline.

CHANGES IN AGGREGATION OF SOIL PARTICLES

The influence of the decomposition of organic matter on soil structure was measured by means of Gerdel's (15) method for determining water-stable aggregates. The results of these analyses are given in Tables 1 and 2; the data for Clarion silt loam, typical of those obtained on all the other soils, are plotted in Figure 4.

In all cases decomposition of the organic materials promoted the formation of larger soil aggregates; this is indicated by the decreasing percentage values for particles less than 0.05 mm. in diameter as incubation proceeded. The formation of larger aggregates continued steadily during the entire course of the experiment, but was most rapid during the first 7 to 15 days of incubation during which organic dissimilation likewise was most rapid. At the end of 133 days, the decrease in particles less than 0.05 mm. in diameter amounted to approximately 27 per cent of the initial values in the Pima clay loam and approximately 40 per cent in the Clarion silt loam.

The nonleguminous material caused greater aggregation of the soil particles than did the legumes in each soil. The order of decreasing effectiveness on aggregation for the four treatments was: Markton oats, hegari, sesbania, sour clover. These findings are in essential agreement
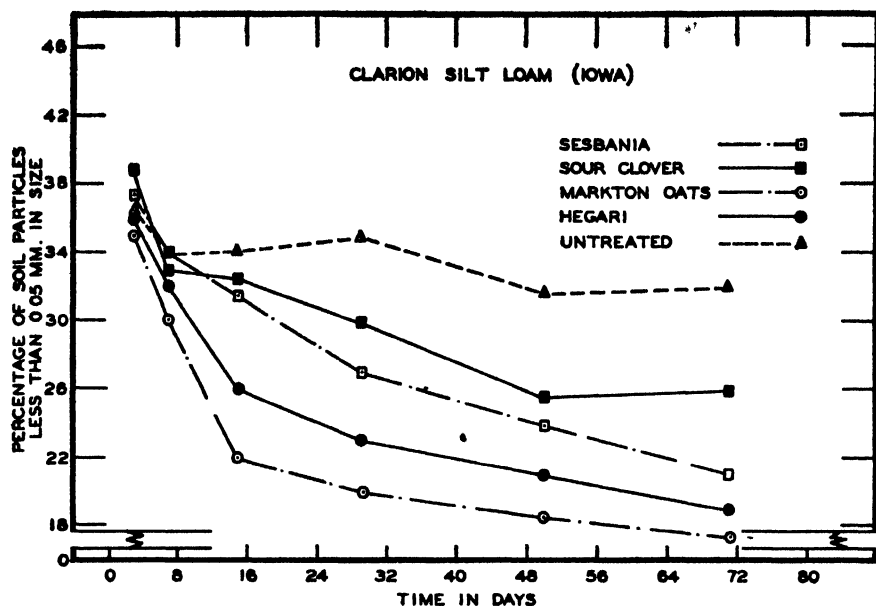
FIG. 4. Changes in size of particles of samples of Clarion silt loam during the decomposition of organic materials.

with those of Chapman (11), that only 10.8 per cent of the soil "crumbs" from a timothy-treated soil would pass through a sixteenth-inch screen, whereas 55.7 per cent of those from a clover-treated soil would do so.

Notwithstanding the normal differences in organic-matter content (Tables 1 and 2) and particle size in the Clarion *silt* loam and the Pima *clay* loam, the decomposition of the added organic materials caused a greater aggregation of the small particles in the Clarion than in the Pima-series soil. Moreover, the difference in influence between any two organic substances was greater in the Clarion than in the Pima soil; for example, after 70 days of incubation the difference in percentage values for particles less than .0.05 mm. in diameter in the sesbania- and in the Markton oats-treated samples was 5 per cent in the Clarion silt loam and only 1 per cent in the Pima clay loam.

No entirely logical explanation for the difference in effect of the legumes and nonlegumes on aggregation has been offered. The chief chemical difference of the two types of plant material is the higher protein content of the sesbania and sour clover (21.8 per cent) as compared with that of oats and hegari (8.5 per cent). Since the rapid conversion of protein-nitrogen to ammonia and then to nitric acid would tend to bring calcium into solution (28), one would expect greater aggregation effects in the legume-treated than in the nonlegume-treated samples of Pima clay loam. Similarly, the inherently higher calcium contents of the leguminous plant materials should show their influence on aggregation, especially in the calcium-deficient Clarion silt loam.

The mechanism of soil aggregation in all of its phases has never been clearly explained. It is known that organic binding materials play an important role in aggregate formation, however, and, according to Peele (31), may be divided into (a) the lyophobic colloids formed as part of the residues of plant materials added to the soil, and (b) microbial cells and their secretory products.

Martin and Waksman (19) showed that bacterial decomposition products are important in aggregate formation, and that microbial gums and mold mycelia are important binding agents. These investigators concluded that the aggregating effect of the various organic materials varied, however, not only with the organisms involved, but also with the nature of the organic material present. The latter appears to be in agreement with the results obtained in the present study. If this were not true, it is unlikely that the different organic materials would have similar aggregating effects in the highly alkaline Pima clay loam and the distinctly acid Clarion silt loam in which it may be assumed that markedly different microflora are present.

It is apparent from the above presentation of results that several of the findings are difficult to explain by existing knowledge in the field. Further study is consequently in prospect and shall be reported upon in subsequent communications.

SUMMARY

1. Data are reported on the influence of the decomposition of sesbania, sour clover, hegari, and Markton oats on the pH, nitrate-nitrogen content, and the degree of aggregation of Pima clay loam, an alkaline-calcareous soil from Arizona, and of Clarion silt loam, an acid soil from Iowa.

2. At a temperature of 30° C. and a moisture content of 65 per cent of the water-holding capacity, the rate of decomposition of each organic material was more rapid in the acid Clarion silt loam than in the alkaline Pima clay loam. At the end of the incubation period, a greater percentage of the nonleguminous than of the leguminous materials had been dissipated with the exception of sour clover; the latter decomposed quickly because of its unusually high content of water-soluble constituents.

3. A pronounced lowering of the pH of Pima clay loam occurred upon the addition of each organic material. Upon decomposition, however, the pH values of all the samples rose quickly and, in the case of the hegari- and oat-treated soils, assumed a position close to that of the untreated samples; this position was maintained essentially during the course of the decomposition. The pH values decreased sharply, coincident with the oxidation of ammonia to nitrates in the sesbania- and sour clover-treated samples.

4. The addition of organic materials was without initial effect on the pH value of Clarion silt loam. Upon decomposition, however, the pH

values of all the samples rose quickly and, in the case of the sesbania- and sour clover-treated samples, assumed a position some 2 pH units above that of the untreated samples. The pH values of the legume-treated samples did not decrease until nitrates began to accumulate and it was not until nearly 500 ppm of nitrate-nitrogen had accumulated (133 days of incubation) that the pH was again equal to that of the un-treated samples. The pH values of the nonlegume-treated samples de-creased more quickly than those treated with leguminous plant materials, but it was not until nearly 95 per cent of the carbon had been dissipated at the end of 133 days of incubation that the pH approached that of the control samples.

5. Nitrates disappeared quickly from both soils during the initial decomposition of the different organic materials. In the hegari- and oat-treated samples, nitrates were present in lesser amounts than in the untreated samples for an incubation period of approximately ten weeks. In the sesbania- and sour clover-treated samples of Pima clay loam, nitrates began to accumulate in one week and over 60 per cent of the nitrogen had been oxidized to nitrates in four weeks. In the sour clover-treated samples of Clarion silt loam, nitrates did not begin to accumulate for four weeks, and in the sesbania-treated, for nearly six weeks. At the end of eight weeks, the nitrate accumulation in the sour clover-treated samples of this soil amounted to only that in the untreated samples.

6. In all cases decomposition of the organic materials promoted the formation of larger soil aggregates; the nonleguminous materials were more effective in promoting aggregation in both soils than were the leguminous materials. The order of decreasing effectiveness in promot-ing aggregation for the four treatments was: Markton oats, hegari, ses-bania, and sour clover. The plant substances had a greater aggregating effect in the *acid* Clarion silt loam than in the *alkaline* Pima clay loam. At the end of 133 days of incubation, the decrease in particles less than 0.05 mm. in diameter amounted to approximately 27 per cent of the initial values in the Pima-series soil, and approximately 40 per cent in the Clarion-series soil.

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A RAPID METHOD FOR THE DETERMINATION OF TOTAL PHOSPHORUS IN SOIL AND PLANT MATERIAL[1]

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Several colorimetric methods have been proposed for the determination of small quantities of phosphorus in biological material. Information concerning many of these procedures may be obtained from a textbook on "Colorimetric Methods of Analysis," by Snell and Snell (10). A recent article by Schricker and Dawson (9) contains a good review of the literature on this subject. A majority of these methods depend upon the partial reduction of molybdenum in phosphomolybdic acid which changes from colorless to blue (7), and the intensity of color is approximately proportional to the quantity of phosphorus in the solution.

One of the important problems in colorimetric determination is to obtain constant-color values in solutions which vary in chemical composition and may contain compounds which interfere with normal color development. Many reducing agents which have been used to determine total phosphorus in biological material by the molybdenum blue method give inaccurate results in the presence of large quantities of ferric iron. Soil extracts from a soil digested with strong acid will frequently contain large quantities of this element. Since ferrous iron does not interfere with the partial reduction of hexavalent molybdenum in phosphomolybdic acid, heating phosphorus solutions containing ferric iron with sodium sulfite has been recommended to change ferric iron to the ferrous form. Truog and Meyer (11) recommend that solutions containing more than 6 ppm of ferric iron should be filtered through a Jones reductor containing cadmium before the molybdenum blue color is developed with stannous chloride. Since this reducing agent forms an unstable molybdenum blue which begins to decompose soon after a maximum intensity of color has developed, it is not as satisfactory for use in routine analysis as other reagents which produce a stable molybdenum blue colloid in solutions containing small quantities of phosphorus.

The upper and lower limits of acid concentration between which the intensity of the molybdenum blue color is relatively constant have been studied by several investigators. Some variation occurs in the quantity of acid recommended because molybdic acid is reduced at a lower acidity by some reducing agents than by others. Schricker and Dawson (9) found that a stable form of molybdenum blue is obtained using metol or

unstable molybdenum blue as a reducing agent when the pH value of a sulfuric acid solution varies from .7 to .83. They recommend the use of quinaldine red as an indicator to aid in the accurate adjustment of acidity in a phosphorus solution before the reducing agent is added. Zinzadze (15) used 2,6-dinitrophenol for this purpose.

EXPERIMENTAL RESULTS

A study of several colorimetric methods recommended for the measurement of small quantities of phosphorus was made to discover or develop a rapid procedure which would be suitable for the determination of total phosphorus in soil and plant material. In the preparation of soil or forage samples for a total phosphorus determination, the organic matter may be destroyed by ignition with or without the addition of an oxidizing agent. Wet ashing with perchloric acid as recommended by King (6) or the use of a perchloric-nitric acid mixture as proposed by Gieseking et al. (4) has many advantages when total ash is not determined. One of the important advantages of the perchloric acid digestion is the elimination of silica without evaporating a solution to dryness, which is required when hydrochloric acid is used to dissolve the phosphorus from plant ash or a soil residue. Willard and Cake (13) found that silica was quickly dehydrated when boiled in concentrated perchloric acid for a short period of time. Volk and Jones (12) used a perchloric-nitric acid mixture for the destruction of organic matter in soil. It was found in this study that pretreatment with nitric acid was necessary only when soil samples containing a large quantity of active organic matter, such as the A_0 layer of a soil profile, a peat, or a muck, were analyzed.

Hillebrand and Lundell (5) report that phosphoric acid was lost by volatilization when a mixture of phosphoric and sulfuric acid was digested in an open platinum dish at temperatures between 200° and 260° for varying periods of time. Concentrated perchloric acid (70-72% $HClO_4$) boils at approximately 203° C. When 4 mg. of phosphorus as monopotassium phosphate was digested with 4 ml. of concentrated perchloric acid in a 30-ml. beaker covered with a watch glass for periods varying from 30 minutes to 4 hours, complete recovery of the phosphorus was obtained. This experiment indicates that perchloric acid may be used safely for the destruction of organic matter in the average soil or plant material without loss of phosphorus by volatilization.

A comparison of several reducing agents which have been used to develop the molybdenum blue color in phosphorus solutions by different investigators (1, 2, 3, 9, 10, 11, 15) indicated that hydrazine sulfate gave the best results when a considerable quantity of ferric iron was present. This reagent has been recommended for the reduction of the molybdenum in phosphomolybdic acid by Riegler (10, 14). Other investigators have used this method (8), and in all cases the ammonium phosphomolybdate precipitate was washed to remove the excess of precipitating reagent be-

fore the molybdenum blue colloid was formed. In this investigation it was found that hydrazine sulfate would not form a blue color with low concentrations of molybdic acid when the pH value of the solution was less than 1.0. If more than 6 ml. of 2 per cent solution of sodium molybdate is added to 150 ml. of solution containing no phosphorus, the pH value of that solution must be less than .85 to prevent color development when boiled with 5 ml. of 2 per cent hydrazine sulfate. Data on the recovery of phosphorus in solutions containing varying quantities of ferric iron using hydrazine sulfate as a reducing agent are given in Table 1. The molybdenum blue color was developed as recommended in the procedure for the analysis of soil. Color intensity comparisons were made with a Cenco-Sheard photelometer.

TABLE 1

THE EFFECT OF FERRIC IRON ON THE RECOVERY OF KNOWN QUANTITIES OF PHOSPHORUS DETERMINED BY THE MOLYBDENUM BLUE METHOD IN 200 ML. OF SOLUTION USING HYDRAZINE SULPHATE AS A REDUCING AGENT

Mg. of Phosphorus Added	Mg of Ferric Iron Added	Mg. of Phosphorus Recovered
.04	0	.040
.10	0	.100
.20	0	.200
.40	0	.400
.50	0	.500
.04	10*	.041
.10	10	.106
.20	10	.200
.40	10	.404
.50	10	.500
.04	25†	.040
.10	25	.111
20	25	.203
.40	25	.404
50	25	.504

* Equivalent to 50 ppm of iron in the solution
† Equivalent to 125 ppm of iron in the solution

Additional studies were also made to determine the effect of 50 mg. of ferric iron on the recovery of phosphorus from solutions containing known quantities of monopotassium phosphate. A slight positive error occurred in the presence of large quantities of iron instead of a negative error which is usually obtained with other colorimetric phosphorus procedures which have been recommended. The positive error is not serious when the total iron content of a soil does not exceed 10 per cent. Under such conditions the total phosphorus in a soil would be increased approximately 32 pounds per acre. Separation of iron from phosphorus may be necessary before the molybdenum blue color is developed in a solution containing a high percentage of iron. Under such conditions the use of a volumetric procedure for total phosphorus would not be objectionable.

When hydrazine sulfate is added to a solution containing molybdic acid and no phosphorus, a pale yellow color will appear when this solution is heated to boiling. This color will interfere with the determination of small quantities of phosphorus if color comparisons are made in a visual colorimeter, because different shades of green are obtained before the blue color produced by increasing quantities of phosphorus will mask the effect of the yellow color appearing in a solution containing no phosphorus. Since a photelometer measures the quantity of light absorbed by a solution rather than the shade or intensity of color which may be present, a variation from the light blue to green colors which are obtained when less than .04 mg. of phosphorus is present in 200 ml. of solution is not objectionable.

Hydrazine sulfate reduces the molybdenum in phosphomolybdic acid very slowly at room temperatures. The rate of reduction increases rapidly between 50° and 70° C. It is necessary to heat a solution containing hydrazine sulfate to boiling in order to reduce ferric to ferrous iron in a short period of time. When a blue color does not appear in solutions which have been boiled for 1 minute and contain phosphorus and ferric iron, more hydrazine sulfate should be added to hasten the rate of reduction. No increase in color intensity occurs when phosphorus solutions are boiled longer than 1 minute after the blue color begins to appear. When green colors are obtained and the reading on the photelometer scale is high, a larger aliquot may be used to increase the accuracy of the determination if iron does not interfere with color development.

A second factor which affects the development of molybdenum blue color is the pH value of the solution in which reduction occurs. When the acidity of the solution is too low, many reducing agents will reduce molybdic acid not combined with phosphorus. When the acidity is too high, a decrease in the intensity of blue color for a known quantity of phosphorus is obtained. Data on the pH value of solutions containing .2 mg. of phosphorus and acidified with varying quantities of sulfuric,

TABLE 2

THE EFFECT OF ADDING VARYING QUANTITIES OF DIFFERENT ACIDS ON THE COLOR INTENSITY OF MOLYBDENUM BLUE PRODUCED BY THE REDUCTION OF .2 MG. OF PHOSPHORUS IN 200 ML. OF SOLUTION WITH HYDRAZINE SULFATE

pH Value of Sulfuric Acid Solutions	Photelometer Readings	pH Value of Hydrochloric Acid Solutions	Photelometer Readings	pH Value of Perchloric Acid Solutions	Photelometer Readings
1.29	58	1.34	58	1.30	57
1.17	58	1.20	59	1.15	61
1.07	59	1.08	60	1.05	60
1.00	58	1.00	60	.96	60
.92	59	.92	60	.90	60
.87	67	.86	60	.84	60
.84	79	.82	61	.80	59
.75	*	.74	72	.72	63
.69	*	.68	86	.66	71

* No blue color developed in these solutions.

hydrochloric, and perchloric acid before the molybdenum blue color was developed with hydrazine sulfate are given in Table 2.

The color values of stable molybdenum blue which are produced with hydrazine sulfate were approximately the same in solutions of similar pH values obtained by the addition of varying quantities of the different acids. Maximum color development appeared in a slightly higher pH range in the presence of sulfuric acid. No color was obtained at the higher intensities of acidity when this acid was used to acidify the phosphorus solutions before the hydrazine sulfate was added. No appreciable difference was observed in the hydrochloric and perchloric acid comparisons. Undesirable colors are obtained when hydrochloric acid is used to dissolve the phosphorus from soil samples which have been ignited to destroy organic matter due to the presence of iron; consequently, perchloric acid has been recommended for the determination of the total phosphorus in soil. Hydrochloric acid may be substituted for the perchloric acid in the analysis of forage.

Molybdic acid is reduced by hydrazine sulfate when boiled in a solution with a pH value above 1.1; consequently, it is necessary. to carefully control the acid concentration to eliminate errors which may occur from the reduction of the precipitating reagent not combined with phosphorus. The photelometer readings of sodium and ammonium molybdate solutions (5 ml. of a 2 per cent solution in 200 ml.) boiled 1 minute with 5 ml. of a 2 per cent solution of hydrazine sulfate in the absence of phosphorus are recorded in Table 3. The pH values were controlled by adding increasing quantities of perchloric acid. The lower the photelometer reading, the greater the quantity of blue color. Only pale yellow colors appeared when the reading was 96 or higher. The blue color in the ammonium molybdate solutions at pH 1.2 and 1.1 was probably due to the slightly larger quantity of molybdic acid which they contained as compared with the sodium molybdate solutions.

TABLE 3

EFFECT OF pH VALUE ON THE REDUCTION OF MOLYBDIC ACID WITH HYDRAZINE SULFATE

REAGENT USED	pH VALUE OF SOLUTIONS AND PHOTELOMETER READINGS							
	1.30	1.20	1.10	1.00	.90	.80	.70	.65
Sodium molybdate ..	74	97	98	98	98	98	98	98
Ammonium molybdate	23	57	93	92	96	96	96	96

The data in Table 3 show that the acidity of a phosphorus solution should be below pH 1.0 in order that no blue color will be formed by the action of hydrazine sulfate on molybdic acid. Six ml. of 2 per cent sodium molybdate will not produce a blue color in the presence of 5 ml. of 2 per cent hydrazine sulfate and 150 ml. of distilled water when the pH of the solution is below 1.0. Since the accuracy of the photelometer is limited by the intensity of the blue color which did not change the light absorp-

tion readings appreciably when more than .6 mg. of phosphorus was present in 200 ml. of solution, more than 5 ml. of 2 per cent sodium molybdate would not be required to precipitate the phosphorus which can be measured by the proposed method. Ten ml. of sodium molybdate will not form a blue color when boiled with hydrazine sulfate if the pH of the solution is less than .85 and the volume is above 175 ml.; consequently, the precipitating reagent can be added rapidly from a pipette or a burette, keeping the volume as near 5 ml. as routine technique will permit.

REAGENTS FOR TOTAL PHOSPHORUS IN SOIL

1. Perchloric acid, 70-72% $HClO_4$
2. Beta dinitrophenol, .1% in 25% alcohol
3. Ammonium hydroxide, 1 part to 3 parts of distilled water
4. Sodium molybdate, 20 gms. of C. P. salt dissolved in 600 ml. of distilled water and add 400 ml. of concentrated perchloric acid (70-72% $HClO_4$) to this solution ($Na_2MoO_4 . 2 H_2O$)
5. Hydrazine sulfate, 20 gms. per liter

RECOMMENDED PROCEDURE FOR TOTAL PHOSPHORUS IN SOIL

Weigh 2 grams of 100-mesh soil into a 30 ml. Pyrex beaker. Add 4 ml. of concentratde perchloric acid (70-72% $HClO_4$) and cover beaker with a small watch glass. Digest on a gas or an electric hot plate covered with an asbestos pad for 30 minutes or until the residue is white. The solution should boil very slowly. When digestion is complete, remove beaker from hot plate, allow to cool a few minutes, wash lower surface of cover glass into the beaker with distilled water, and transfer the contents to a 200 ml. calibrated Erlenmeyer flask using a funnel with a short stem. Add distilled water to the mark, place a rubber stopper in the flask, shake thoroughly, and filter to remove the insoluble residue. Pipette 50 ml. of the filtrate which should contain from .04 to .6 mg. of phosphorus into another 200 ml. calibrated Erlenmeyer flask. Add approximately 100 ml. of distilled water and 5 drops of 2,6-dinitrophenol. Titrate the acidity in this solution with ammonium hydroxide until the yellow color of the indicator appears. Add 5 ml. of the sodium molybdate reagent, 5 ml. of hydrazine sulfate, place the flask on a hot plate, heat to boiling, and allow to boil about 1 minute after the blue color begins to appear. If a blue color does not appear within 1 minute after the solution starts to boil, add more hydrazine sulfate and continue heating. Remove flask from hot plate, cool to room temperature, add distilled water to the 200 ml. mark, mix solution thoroughly, and obtain a light absorption reading with a photelometer. The quantity of phosphorus in the soil is obtained from a curve prepared by plotting the photelometer readings of several phosphorus solutions containing from .025 to .750 mg. of phosphorus on cross-section paper. If a visual colorimeter is used, at least three standard solutions containing small, medium, and large quantities of phosphorus

should be prepared in order that more accurate color comparisons with the unknown solutions may be obtained.

In the analysis of peat, a .5-gram sample should be digested in a 200 ml. tall-form Pyrex beaker with 8 or 10 ml. of the nitric-perchloric acid mixture as recommended for forage and grain. Soils containing a high percentage of iron and/or manganese can be digested more rapidly by adding a small quantity of sodium chloride. Soils containing a high percentage of clay may require more than 4 ml. of perchloric acid for digestion.

RECOMMENDED PROCEDURE FOR TOTAL PHOSPHORUS IN FORAGE OR GRAIN

Place 1 gram of finely ground oven-dry forage or grain in a 200 ml. tall-form Pyrex beaker. Add 7 ml: of acid containing 1 part of perchloric ($70-72\%$ $HClO_4$) and 1 part of concentrated nitric acid. Cover beaker with a watch glass and digest on a steam plate or hot water bath for 20 minutes. Transfer beaker to a gas or electric hot plate covered with an asbestos pad, remove cover glass to permit nitric acid to escape, and heat gently until white fumes of perchloric acid have evolved for 1 minute. Replace cover glass and continue heating until the solution is colorless and the residue is white. Remove beaker from hot plate, cool, and transfer solution and residue to a calibrated 200 ml. Erlenmeyer flask, and add distilled water to the mark. Stopper flask, mix contents thoroughly, and filter to remove insoluble residue. Transfer 25 ml. of the filtrate to a calibrated 200 ml. Erlenmeyer flask and develop the molybdenum blue color as recommended for soil. A larger aliquot should be taken if the sample is low in total phosphorus. A smaller aliquot should be used if organic materials high in total phosphorus are being analyzed. If a larger sample is needed for the determination of other elements in the forage or grain, more acid should be added to destroy the organic matter. If the forage or grain is ignited to destroy organic matter, the ash may be treated with perchloric acid to remove silica and the phosphorus determined in a convenient aliquot by the recommended procedure. Hydrochloric acid may be used in place of the perchloric acid to extract the phosphorus from the ignited residue since the iron content of forage samples is usually low.

Molybdenum blue colors which are formed when phosphomolybdic acid is reduced with hydrazine sulfate are very stable and remain constant for a long period of time. In routine analyses, it is convenient to develop the color in an aliquot and allow the solution to cool overnight before the photelometer reading is obtained. A comparison of photelometer readings obtained at intervals of 2 and 16 hours after the blue color of phosphomolybdic acid had been developed is given in Table 4. These analyses were made in quadruplicate on two soils to show the variations that may be expected between individual analyses.

It will be observed that no appreciable change in the color of these solutions occurred during the 14-hour period as measured by the photelometer. The greatest variation between the photelometer readings for

TABLE 4

| Soil No. | Sample No. | PHOTELOMETER READINGS | |
		2 Hours After Color Development	16 Hours After Color Development
4193	1	57.0	56.8
	2	58.0	57.8
	3	57.9	57.9
	4	57.8	57.6
5174	1	71.0	71.0
	2	70.4	70.4
	3	70.0	70.0
	4	70.5	70.5

each analysis was one division on the photelometer scale, which is equivalent to 28 pounds of phosphorus per acre 6⅔ inches deep in sample 4193, and 24 pounds per acre in sample 5174. Since the readings between each division on the photelometer scale must be estimated, a difference of .2 cannot be considered significant.

A comparison of the total phosphorus in 206 soils analyzed by the colorimetric method described in this paper and by two volumetric procedures was made to determine whether similar results would be obtained. Eighty-four of these soils were analyzed in 1926 by the magnesium nitrate fusion method. One hundred and twenty-two samples were analyzed within the past three or four years by the method as recommended by Volk and Jones (12). The average phosphorus content of the 206 soil samples as determined by the two volumetric methods was 408 pounds per acre. The average phosphorus content of these samples when analyzed by the recommended colorimetric procedure was 407 pounds per acre. Only 27 of the 206 samples had a wider variation than 40 pounds of total phosphorus per 2 million pounds of soil. Of this number, 15 of the samples were higher and 12 samples were lower in total phosphorus content as determined by the volumetric procedures. Since different methods of digestion were used in these analyses, some difference in lack of agreement might be expected.

A comparison of the variation which might occur in the results obtained by different individuals using the colorimetric procedure as recommended was secured on 60 samples of soil analyzed by two individuals with a time interval between these analyses of approximately one year. The average total phosphorus content in these two sets of analyses varied less than .001 per cent. The greatest variation between individual samples of soil was .006 per cent or 120 pounds of phosphorus per acre 6⅔ inches deep. This variation would probably be greater if soils containing a larger quantity of total phosphorus were analyzed. The average total phosphorus content of the 60 soils was approximately .02 per cent and varied from .01 to .039.

During the past two years several thousand samples of soil, forage, and grain have been analyzed for total phosphorus by this method. The important advantage of this procedure as compared with a volumetric or a gravimetric determination is that less time is required to make the analyses. The cost of expendible material for each soil analysis is approximately 2 cents. The accuracy of the proposed colorimetric method is not the same at all points on a curve prepared by plotting photelometer readings against the quantity of phosphorus in standard phosphorus solutions. Less variation will occur between the photelometer readings of duplicate or triplicate analyses when smaller quantities of phosphorus are present. The standard phosphorus solutions which were used to prepare a curve from which the phosphorus in an unknown solution could be calculated were prepared from monopotassium .phosphate diluted to a volume of 200 ml. before the readings were obtained. The phosphorus in the standard solutions varied from .025 to .75 mg. 'Photelometer readings obtained after the molybdenum blue color was developed in the phosphorus solutions are given in Table 4. A reading of 98 which was obtained when no phosphorus was added represents a blank determination on the reagents.

TABLE 5

PHOTELOMETER READINGS OF STANDARD PHOSPHORUS SOLUTIONS IN WHICH THE MOLYBDENUM BLUE COLOR WAS DEVELOPED WITH HYDRAZINE SULFATE

Mg. of Phosphorus in Standard Solutions	Photelometer Readings
.000	98.0
.025	92.0
.050	86.1
.100	76.0
.150	67.8
.200	60.0
.250	53.2
.300	47.5
.350	42.3
.400	38.5
.450	34.5
.500	31.0
.550	28.3
.600	26.5
.650	25.5
.700	25.6
.750	25.2

It is evident from the data presented in Table 4 that the accuracy of a phosphorus determination will decrease with increasing quantities of phosphorus in solution. When more than .6 mg. of phosphorus is present in 200 ml. of solution, a smaller aliquot should be used. Since very few soils contain more than 2,400 pounds of total phosphorus in the surface layer 6⅔ inches deep, no changes in the recommended procedure will be required for the average soil. If the logarithm of each photelometer reading is plotted on cross-section paper against the quantity of phosphorus in

the standard solutions as given in Table 4, a slight break will be observed in the curve obtained indicating that the intensity of color is not exactly proportional to the amount of phosphorus present. The tendency for the colloidal particles to aggregate as the concentration of phosphorus in a solution is increased may account for this condition, although studies on the dilution of standard phosphorus solutions with equal quantities of distilled water after color development has occurred has not had any appreciable effect on the recovery of phosphorus in them.

SUMMARY

A study was made of several colorimetric methods recommended for the determination of phosphorus in different types of organic materials to ascertain their value for the estimation of total phosphorus in soil. Large quantities of ferric iron retard the rate at which the molybdenum blue color is formed when reducing agents recommended for this purpose were used. Hydrazine sulfate will produce a stable molybdenum blue which does not change appreciably after color development has occurred. This reagent will reduce ferric iron at the boiling temperature, and reasonably accurate results for total phosphorus will be secured if the total iron in a soil does not exceed 10 per cent. Hydrazine sulfate does not produce a blue color when added to a solution containing less than .12 mg. of sodium molybdate in 200 ml. of distilled water if the pH value of the solution is properly controlled and no phosphorus is present. A colorimetric method using this reagent to form a stable molybdenum blue colloid from phosphomolybdic acid has been proposed for soils which do not contain more than 10 per cent of iron. The total phosphorus in 206 samples of soil determined by precipitation and subsequent titration of ammonium phosphomolybdate was compared with results obtained by the proposed colorimetric method. Only 27 of the 206 analyses varied more than .002 per cent in total phosphorus content by the volumetric and colorimetric procedures. The method for total phosphorus in soil can also be used for the determination of total phosphorus in forage and grain by using a mixture of nitric and perchloric acid to destroy the organic matter in these materials.

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STUDIES OF DIFFERENT CULTURES OF *RHIZOBIUM LEGUMINOSARUM* AND OF GYPSUM AND STRAW FOR SEED PEA PRODUCTION[1]

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INTRODUCTION

The production of seed peas and wheat in alternate years is common practice in those parts of eastern Washington and northwestern Idaho where the annual precipitation is more than 18 inches. According to Rufener (12) this area, comprising approximately 960,000 acres, nearly two-thirds of which is situated in the State of Washington, produces more than half of the total amount of seed peas harvested in the United States. As a source of agricultural income, seed peas in this area rank second to wheat, the principal crop.

When peas were first introduced as a field crop on Palouse silt loam, seed inoculation with cultures of *R. leguminosarum* was necessary to obtain satisfactory growth and yields. As shown by earlier work (14), once this soil is thoroughly inoculated with *R. leguminosarum*, this organism is capable of surviving in the soil for 10 to 15 years in the absence of the host plant. Reinoculation, therefore, after one thoroughly inoculated crop of peas has been produced is unnecessary insofar as satisfactory production of root nodules is concerned. Since the variability in the ability of different strains or cultures of *Rhizobium* species to benefit their homologous host plants has been demonstrated convincingly by various investigators, notably Baldwin and co-workers (1, 2, 4, 5, 7) and others (8, 9, 11, 15), it was thought that this factor might influence the degree by which seed peas can be benefited from association with their homologous symbiotic nitrogen-fixing bacteria. Further development of this thought found additional support in the fact that in this area where soil moisture and available nitrogen are limiting factors in crop yields in annual cropping systems, seed peas fill a place which cannot be filled by nonleguminous crops. Although the yield of wheat following peas is less than that follow-

ing summer fallow, it is much larger than that following nonleguminous crops. Deficiency in available nitrogen is one of the principal factors causing reduction in yields in pea-wheat rotations. When both the straw and the stubble of the wheat crop are incorporated in the soil, the nitrogen deficiency is accentuated. Bjälfve (3) and Löhnis (9) noted, however, that symbiotic nitrogen fixation by the pea-nodule bacteria was enhanced in culture media that are poor in available nitrogen. Possibly the deficiency in available nitrogen prevailing in pea-wheat rotations can be reduced in intensity if symbiotic nitrogen fixation can be increased by more effective root-nodule bacteria.

Applications of sulfur or gypsum for alfalfa on Palouse silt loam have been known to result in better growth and larger yields. Neller (10) found evidence of this fact in pot cultures in the greenhouse, but obtained irregular results in plot experiments under field conditions. He attributed part of the beneficial effect of sulfur or gypsum on alfalfa to the stimulative action of sulfur on the activity of the nodule bacteria. The possibility that sulfur compounds may produce similar effects on nodule bacteria of peas is worthy of consideration.

The chief purpose of the studies reported in this paper was to determine the effect of different cultures of R. leguminosarum and of applications of gypsum and wheat straw on the growth and yield of seed peas on Palouse silt loam.

EXPERIMENTAL PLANS AND METHODS

Palouse silt loam is a fertile grassland soil. The topography of the seed pea-producing area is hilly and the slopes vary in productivity, depending in a large measure upon the depth of the dark brown topsoil. In virgin soils this depth ranges from less than 8 inches on certain hilltops to more than 20 inches on the lower slopes. The slope, size, and shape of the experimental field available for this study are such that the depth of the dark brown topsoil ranges from less than 6 inches to about 18 inches. This situation has the advantage of representing actual farming conditions and the disadvantage of presenting wide variations in soil productivity.

Insofar as is known, peas had never been produced in the experimental field, but occasional volunteer hairy vetch plants have been found growing there every year for the last 20 years. The inoculation experiment was repeated for three years in succession, and the experimental plots which varied from 1/150 to 1/200 acres in different years, depending upon the availability of suitable space, were located in a new area in the field each year. Plots with the same inoculation treatment were replicated three times and so distributed in the field that as near as possible one plot occupied an area where the depth of topsoil was less than 6 inches, another an area where the depth of topsoil was from 6 to 12 inches, and the third an area where the depth of topsoil exceeded 12 inches.

Alaska peas served as the experimental crop which was produced on

plowed wheat stubble land, except in 1939 when stubble land was not available and summer-fallow land was used instead. The peas were seeded with a "Planet Junior" seeder in rows 9 inches apart at the rate of 160 pounds per acre, a somewhat larger rate than the usual field rate, which is 120 pounds per acre. The seed was inoculated with the various cultures of *R. leguminosarum* in accordance with the methods recommended for field practice with the exception that the concentration of the suspensions was somewhat larger. Aseptic precautions were observed in the inoculation procedure, and those parts of the "Planet Junior" seeder which came in contact with the seed were washed thoroughly with 75 per cent ethyl alcohol each time a lot of peas inoculated with a different culture was introduced. Yields were obtained from three 1-square-yard quadrates harvested from each plot.

The effect of applications of gypsum and of wheat straw to the soil was determined in a pot culture experiment in the greenhouse. Wheat straw treatments were included because in many cases in field practice the straw and stubble of the previous wheat crop are incorporated in the soil before the peas are planted. The soil used for this experiment was taken from the surface 8-inch layer in a field where the topsoil was about 12 inches deep and which had been in a pea-wheat rotation for about 30 years. All the treatments were replicated twice. The wheat straw which was finely ground was mixed with the surface 3 inches of soil, and the gypsum was applied with the pea seed which was planted in two rows in each pot to simulate field planting. The variety of peas was the same as that used for the field plots.

EFFECT OF DIFFERENT CULTURES OF *R. LEGUMINOSARUM* ON NODULATION AND YIELD OF PEAS

The inoculation treatments of the small experimental plots of Alaska peas produced during 1938, 1939, and 1940, and the average yields obtained from three separate square-yard quadrates are given in Table 1.

The most striking characteristic of the yield data is their irregularity as shown in the columns from left to right by different plots planted to peas that received the same inoculation treatment. It is evident, also, that in general and regardless of inoculation treatments the plots where the dark brown topsoil was less than 6 inches in depth produced considerably lower yields than those where the topsoil layer was between 6 and 12 inches thick, and much lower than the plots where the topsoil layer was more than 12 inches thick. The extremely small yields on plots 4, 7, and 8 in 1939 are explainable by the fact that they occupied a typical clay hill knoll where the topsoil was very shallow or nonexistent. In interpreting the experimental results from the standpoint of the effectiveness of different cultures of pea-nodule bacteria, the significant difference in soil productivity based on thickness of the dark-colored topsoil layer and convincingly demonstrated by differences in yield of seed peas as indicated by analysis of variance introduced a disturbing factor. Soil variability

TABLE 1

Yields from Three 1-Square-Yard Quadrates of Alaska Peas Inoculated with Mixed and Single Physiological Strains of R. Leguminosarum

Treatment	R. leguminosarum Culture	Topsoil Depth Less Than 6 in.				Topsoil Depth 6 to 12 in.				Topsoil Depth More Than 12 in.				3-year Ave.	Increase Over Non-Inoculated Plots
		1938	1939	1940	Ave.	1938	1939	1940	Ave.	1938	1939	1940	Ave.		
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
1	No Inoculation	204	205	230	213	254	216	242	237	280	303	265	283	244	
2	S.C.W.*	279	201	245	242	307	252	260	273	313	296	325	311	275	31
3	L.A.*	263	169	242	225	281	290	287	286	290	297	380	322	278	34
4	N.X.*	271	82	225	193	304	240	317	287	354	415	337	369	283	39
5	W. 317†	238	132	250	207	250	249†	335	278	298	353	345	332	272	28
6	W. 312†	219	111	220	183	261	296	342	300	316	417	345	359	281	37
7	3 HOQ 12†	233	82	280	198	253	213	305	257	306	357	332	332	262	18
8	3 HOQ 9†	269	91	260	207	272	235	290	266	287	302	325	305	259	15

* Commercial culture.
† Single physiological strain.
‡ Filled in by the method of Yates (13).

Analysis of Variance*

Source of Variation	Degree of Freedom	Mean Square
Total—Soil groups	23	
Topsoil Depths	2	84,080†
Strain	7	1,551
Interaction (error)	14	1,500

* For significance (5 per cent level) the difference in values required between any two means of cultures or strains is .41.
† Highly significant.

in this case, however, should be considered as a logical and proper part of the experiment, for it represents normal field conditions on the farms in the seed pea-producing area.

Nitrogen deficiency is one of the chief causes of small crop yields on the hilltops and upper slopes. Good crops of wheat can be produced on them when adequate amounts of nitrogen are supplied in the form of nitrogen fertilizers. It might be assumed that with an adequate supply of soil moisture, thoroughly inoculated peas on these soils should be capable of fixing enough atmospheric nitrogen to produce optimum growth and yields, for plant nutrients other than nitrogen do not appear to be deficient, and in years of normal precipitation the supply of soil moisture is not seriously inadequate, as has been shown repeatedly by luxuriant growth of wheat. Bjälfve (3), using quartz sand and soil poor in nitrogen as media in pot cultures for field peas, found that well-inoculated peas grew excellently on the nitrogen they fixed symbiotically. Even though it is not likely that soil moisture or essential plant nutrients other than nitrogen were seriously deficient in the plots with shallow topsoil in our experiment, Bjälfve's results were not verified, for the growth of the peas was poor and the yields were low. Apparently, not enough nitrogen was fixed symbiotically to insure luxuriant growth.

Attempts have been made by various investigators to correlate the effectiveness of different strains or culture selections of rhizobia with the number, distribution, and size of the root nodules of the homologous host plants, but the results were not uniform. Ruf and Sarles (11) contended that effective strains of R. japonicum produced a few large nodules near the surface of soybean roots, whereas ineffective strains produced many scattered, small nodules. Erdman and Wilkins (6) found that the percentage of nitrogen fixed by soybeans increased with increasing nodulation of the plants. The work of Baldwin and Fred (2) with various strains of R. trifolii revealed that more nodules were formed by the poor strains, but the nodules were small and distributed over the entire root system. Later Baldwin (1) asserted that the number of nodules is not indicative of their beneficial effect on the host plant, although greenhouse experiments indicated that a few large nodules near the top of the taproot gave the greatest benefit, and parasitic strains produced many scattered nodules.

Careful examinations made each year at different growth stages of the Alaska peas in the various plots in our experiment disclosed that although the plants inoculated with different cultures of R. leguminosarum sometimes showed apparent differences in size, or distribution, or number of root nodules in the early growth stages, these differences were not stable, as they could not be verified by subsequent examinations at more advanced growth stages. The peas in all the plots, including those produced by noninoculated seed, had an abundance of root nodules and no significant permanent differences were exhibited with respect to size, distribution, and number of nodules.

As may be noted from the data in Table 1, seed inoculation had a tendency to result in increased yields of seed peas. Statistical treatment of the yield data as indicated by the analysis of variance shows that distinct differences in yields of seed peas resulted from variability in soil productivity, and that the beneficial effect caused by inoculation should be represented by a mean increase in yield of 41 grams in order to be significant. This value was closely approached by two of the seven cultures used, one, N. X., being a commercial culture probably composed of mixed strains of organisms, and the other, W. 312, a single physiological strain. The difference in effectiveness of the various cultures used for inoculation was not significant, but the *R. leguminosarum* organisms present in the soil and probably maintained in active state from year to year by the few scattered vetch plants proved to be less effective in benefiting Alaska seed peas.

EFFECT OF WHEAT STRAW AND GYPSUM ON THE GROWTH OF PEAS

The 1-gallon pots used in this experiment were filled with equal amounts of Palouse silt loam taken from the field described in the experimental plan. Excessively large applications of wheat straw were made in order to reduce the supply of available nitrogen in the soil to a minimum and determine the effect of this factor on the growth of the peas. Gypsum was applied at different rates and placed in contact with the seed in the seed rows to ascertain if larger quantities than those used and drilled in with the seed in field practice would affect germination. The same number of seeds which were inoculated with one of the commercial cultures used in the field plots were planted in each pot, and after emergence the plants were thinned to 12 per pot. The tops and roots were harvested separately at the time vegetative growth ceased. The rates of application of straw and gypsum, and the weights of dry matter of tops and roots of the peas are recorded in Table 2.

TABLE 2

YIELDS OF DRY MATTER OF TOPS AND ROOTS OF ALASKA PEAS GROWN IN 1-GALLON POTS ON PALOUSE SILT LOAM TREATED WITH WHEAT STRAW AND GYPSUM

Pot No	Treatments	Rate per Acre	Tops	Ave	Roots	Ave
		lbs	gms.	gms.	gms.	gms.
3 & 4	Check		28.1		3.1	
			13.7	20.9	2.3	2.7
5 & 6	Wheat straw ..	20,000	18.4		3.4	
			17.5	18.0	2.9	3.3
7 & 8	Wheat straw ..	20,000	13.6		2.1	
	Gypsum	200	16.6	15.1	2.7	2.4
9 & 10	Gypsum	100	16.9		2.7	
			15.4	16.2	2.0	2.4
11 & 12	Gypsum	200	19.4		2.6	
			11.8	15.6	2.0	2.3
13 & 14	Gypsum	400	20.0		2.4	
			21.2	20.6	2.7	2.6

Close observations made of the plants at progressive stages of maturity disclosed that germination was excellent and unaffected by any of the soil treatments. All plants, regardless of treatment, had an abundance of well-developed nodules on their roots. At the bloom stage the plants in the straw-treated pots were less vigorous and slightly less green in color than those in the other pots, particularly those in the pots treated with 200 and 400 pounds of gypsum per acre. At the time of harvest the plants in the straw-treated pots were more advanced in maturity than those in the other pots. The gypsum treatments had a tendency to prolong the period of active growth.

As may be noted from the yield data, the amount of dry matter produced by the tops and roots of the peas varied considerably regardless of treatments. Because of these variations the experimental data may not be conclusive, but it seems that the apparent differences in yield resulting from any of the treatments are insignificant. If stimulation of symbiotic nitrogen fixation occurred at all as a result of applications of gypsum or of reduced quantities of available soil nitrogen caused by the addition of large quantities of straw to the soil, it was insufficient in amount to effect significant increases in the production of vegetative matter of the peas.

Although apparently better yields of peas in the field plots were obtained from inoculated seed than from noninoculated seed, they were not significant statistically, and the nodulation and yield data did not indicate any significant difference in the effectiveness of seven selected cultures of R. leguminosarum to benefit the peas. Additions of straw and gypsum to the soil did not seem to be significant factors in influencing the effectiveness of one of these cultures. It would seem, therefore, that under the prevailing practice of growing peas and wheat in alternate years on Palouse silt loam in the seed pea-producing area, seed inoculation is not likely to be greatly beneficial once the soil has been thoroughly inoculated with an effective culture of R. leguminosarum, unless further investigation reveals that repeated passage of this organism through the same host plant reduces its beneficial effectiveness.

SUMMARY

A study was made to determine the effect of seven different cultures of R. leguminosarum on the nodulation and yield of Alaska seed peas grown three years in succession in small field plots on Palouse silt loam which had not produced peas before. One of these cultures was used to determine the influence of applications of large quantities of wheat straw and of different amounts of gypsum to the soil in pot cultures in the greenhouse on the effectiveness of the culture in benefiting the host plant.

The soil in the experimental field was found to contain a sufficient number of R. leguminosarum to effect thorough inoculation of the peas. Seed inoculation with seven different cultures had a tendency to cause increases in yields of seed peas. Two of these cultures, a commercial culture and a single physiological strain, resulted in increased yields that approached significance statistically.

Significant differences in yield of peas occurred as a result of variability in soil productivity, caused in a large measure by differences in depth of dark brown topsoil and in the supply of available nitrogen.

None of the cultures was sufficiently effective in symbiotic nitrogen fixation to insure vigorous growth of the peas on soil which was low in available nitrogen, and no significant difference was observed in the number, size, and distribution of the root nodules regardless of inoculation treatments.

The large quantities of straw applied to the soil in pot cultures in the greenhouse and the different amounts of gypsum placed in contact with the seed had no detrimental effect on seed germination. These treatments had no marked influence on the effectiveness of the *R. leguminosarum* culture used for seed inoculation either in producing root nodules or in benefiting the growth of the host plant.

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AN IDENTIFICATION SCHEME FOR NUMBERING CULTURES OF *RHIZOBIA*

LEWIS W. ERDMAN[1]

The accumulation of large numbers of cultures of *Rhizobia* isolated from different genera, species, and varieties of legumes presents a real problem in the absence of a well-planned system for numbering individual cultures. Some scheme for labeling *Rhizobia* cultures to reveal their immediate identity with respect to source history and cross-inoculation group affiliation would seem to be highly desirable and almost necessary where large numbers of cultures are kept in stock. Such a scheme would prove especially valuable if adopted universally by the various laboratories and institutions working with these bacteria.

The purpose of this paper is to offer an identification scheme which the author believes would simplify and standardize the labeling of legume bacteria cultures. The idea for this scheme originated while working on the Iowa Agricultural Experiment Station project, "Studies on Rhizobium".[2] One phase of this project included the isolation and testing of cultures of *Rhizobia* from nodules of legumes widely separated botanically. An outline of the family *Leguminosae* was drafted with particular emphasis at that time being placed upon available species of the different cross-inoculation groups found growing in Iowa soils.[3] The books of Bailey (1), Britton and Brown (2), Engler and Prantl (3), Asa Gray (5), and Piper (6) were used as texts in working up the outline. The family *Leguminosae* is divided into three subfamilies, *Mimosoideae*, *Caesalpinioideae*, and *Papilionatae*. These subfamilies are subdivided into tribes, and many tribes in the *Papilionatae* are further divided into subtribes. Under the tribes and subtribes are listed the species of legumes. The scheme of numbering and lettering of the various subdivisions is shown in the outline given below. Where no subtribe is given the capital letter O is inserted.

FAMILY *LEGUMINOSAE*

1. Subfamily MIMOSOIDEAE
   A. Tribe *Acacieae*
      O. a. *Acacia filiculoides* (Prairie acacia)
         b. *Acacia armata*
         c. *Acacia linifolia*

B. Tribe *Eumimoseae*
    O. a. *Mimosa pudica* (Cultivated sensitive plant)
       b. *Acuan illinoiensis (Desmanthus brachylobus)*
       c. *Morongia uncinata (Schrankia uncinata)*
2. Subfamily CAESALPINIOIDEAE
  A. Tribe *Bauhinieae*
    O. a. *Cercis canadensis* (Red bud)
  B. Tribe *Cassieae*
    O. a. *Cassia chamaecrista (fasciculata)* (Partridge pea)
       b. *Cassia marilandica* (Wild senna)
       c. *Cassia nictitans*
  C. Tribe *Kramerieae*
    O. a. *Krameria secundiflora*
  D. Tribe *Eucaesalpinieae*
    O. a. *Gleditschia triacanthos* (Honey locust)
       b. *Gleditschia aquatica*
       c. *Gymnocladus dioeca* (Kentucky coffee tree)
       d. *Hoffmanseggia jamesii*
3. Subfamily PAPILIONATAE
  A. Tribe *Sophoreae*
    O. a. *Sophora sericea* (Silky sophora)
       b. *Cladrastis lutea* (American Yellow Wood)
  B. Tribe *Podalyrieae*
    O. a. *Thermopsis rhombifolia* (Prairie Thermopsis)
       b. *Baptisia tinctoria* (Wild indigo)
       c. *Baptisia bracteata* (Large bracted wild indigo)
       d. *Baptisia leucantha*
       e. *Baptisia australis*
       f. *Baptisia alba*
  C. Tribe. *Genisteae*
    1. Subtribe *Crotolariinae*
       a. *Crotolaria sagittalis* (Rattle Box)
       b. *Crotolaria juncea* (Sunn hemp)
       c. *Crotolaria capensis*
       d. *Crotolaria retusa* (Cherokee clover)
       e. *Crotolaria candicans*
       f. *Crotolaria spectabilis*
    2. Subtribe *Spartiinae*
       a. *Lupinus argenteus* (Silvery lupine)
       b. *Lupinus albus* (White lupine)
       c. *Lupinus perennis* (Wild lupine)
       d. *Lupinus luteus* (Yellow lupine)
       e. *Lupinus angustifolius* (Blue lupine)
       f. *Lupinus polyphyllus*
       g. *Lupinus hirsutus*
       h. *Lupinus texensis* (Texas blue bonnet)

3. Subtribe *Cytisinae*
   a. *Ulex europaeus* (Thorn Broom)
   b. *Cytisus scoparius* (Scotch Broom)
D. Tribe *Trifolieae*
   O. a. *Medicago sativa* (Alfalfa or Lucerne)
      b. *Medicago lupulina* (Black Medic)
      c. *Medicago arabica* (Southern Giant Bur clover)
      d. *Medicago hispida* (Bur clover)
      e. *Medicago arabica* var. (Manganese Bur clover)
      f. *Medicago orbicularis* (Button clover)
      g. *Melilotus indica* (Annual yellow sweet clover)
      h. *Melilotus alba* (Biennial white sweet clover)
      i. *Melilotus officinalis* (Yellow sweet clover)
      j. *Melilotus alba* (an.) (Hubam clover)
      k. *Trifolium pratense* (Red clover)
      l. *Trifolium hybridum* (Alsike clover)
      m. *Trifolium repens* (White clover)
      n. *Trifolium repens* var. (Ladino clover)
      o. *Trifolium agrarium* (Yellow or hop clover)
      p. *Trifolium procumbens* (Smaller hop clover)
      q. *Trifolium incarnatum* (Crimson clover)
      r. *Trifolium alexandrinum* (Berseem clover)
      s. *Trifolium reflexum* (Buffalo clover)
      t. *Trifolium glomeratum* (Cluster or McNeill clover)
      u. *Trifolium resupinatum* (Persian clover)
      v. *Trifolium fragiferum* (Strawberry clover)
      w. *Trifolium subterraneum* (Subterranean clover)
      x. *Trifolium dubium* (Little hop clover)
E. Tribe *Loteae*
   O. a. *Lotus corniculatus* (Bird's-foot trefoil)
      b. *Lotus americanus (Trigonella Foenum-Graecum)* (Fenugreek)
      c. *Lotus uliginosus*
      d. *Lotus tetragonolobus* (Square pod pea)
      e. *Anthyllis vulneraria* (Kidney vetch)
F. Tribe *Galegeae*
   1. Subtribe *Indigoferinae*
      a. *Cyamopsis tetragonoloba* (Guar)
      b. *Indigofera leptosepala* (Wild indigo)
      c. *Indigofera tinctoria*
   2. Subtribe *Psoraliinae*
      a. *Parosela enneandra*
      b. *Parosela dalea (Dalea alopecuroides)*
      c. *Amorpha canescens* (Lead plant)
      d. *Amorpha fruticosa* (False or Bastard indigo)
      e. *Amorpha nana* (Fragrant false indigo)

    f. *Kuhnistera (Petalostemom) candida*
    g. *Kuhnistera purpurea*
    h. *Psoralea tenuiflora* (Few-flowered psoralea)
    i. *Psoralea floribunda* (Many-flowered psoralea)
    j. *Psoralea argophylla* (Silver-leaf psoralea)
3. Subtribe *Tephrosiinae*
    a. *Galega officinalis* (Goat's rue)
    b. *Tephrosia (Cracca) virginiana*
    c. *Kraunhis (Wisteria) frutescens*
4. Subtribe *Robiniiae*
    a. *Sesbania macrocarpus* (Long-podded sesbania)
    b. *Robinia pseudacacia* (Black locust)
    c. *Robinia hispida* (Rose acacia)
    d. *Robinia viscosa*
5. Subtribe *Coluteinae*
    a. *Swainsona* sp.
6. Subtribe *Astragalinae*
    a. *Astragalus rubyi* (Green)
    b. *Astragalus falcatus*
    c. *Phaca neglecta* (Cooper's milk vetch)
    d. *Oxytropis (Spesia) (lamberti)* (Crazy weed)
    e. *Glycyrrhiza lepidota* (Wild liquorice)
    f. *Glycyrrhiza glabra*
    g. *Caragana arborescens* (Siberian pea tree)
    h. *Astragalus sinicus*
    i. *Astragalus canadensis* (Cow vetch)
G. Tribe *Hedysareae*
1. Subtribe *Coronillinae*
    a. *Ornithopus sativa* (Seradella)
    b. *Coronilla varia* (Axseed) (Crown vetch)
2. Subtribe *Euhedysarinae*
    a. *Hedysarum coronarium* (Spanish sanfoin)
    b. *Hedysarum americanum*
    c. *Onobrychis sativum* (Sanfoin)
    d. *Onobrychis viciaefolia*
3. Subtribe *Aeschynomeninae*
    a. *Aeschynomene virginica* (Sensitive joint vetch)
4. Subtribe *Stylosanthinae*
    a. *Stylosanthes biflora* (Pencil flower)
    b. *Arachis hypogaea* (Peanut)
5. Subtribe *Desmodiinae*
    a. *Desmodium purpureum* (Florida beggarweed)
    b. *Meibomia grandiflora*
    c. *Meibomia canescens*
    d. *Meibomia paniculata*
    e. *Meibomia illinoensis*

  f. *Lespedeza hirta* (Hairy bush clover)
  g. *Lespedeza striata* (Common Japan clover)
  h. *Lespedeza capitata*
  i. *Lespedeza stipulacea* (Korean)
  j. *Lespedeza serecia*
  k. *Lespedeza kobe*
  l. *Lespedeza virginia*
  m. *Lespedeza juncea*

H. Tribe *Vicieae*
  O. a. *Cicer arietinum* (Chick pea)
   b. *Vicia americana* (American vetch)
   c. *Vicia hirsuta* (Hairy vetch)
   d. *Vicia* sp. (Calcarata vetch)
   e. *Vicia caroliniana* (Carolina vetch)
   f. *Vicia sativa* (Common vetch)
   g. *Vicia faba* (Broad bean)
   h. *Vicia angustifolia* (Narrow-leaved vetch)
   i. *Vicia atropurpurea* (Purple vetch)
   j. *Vicia monantha* (Monantha vetch)
   k. *Lathyrus odoratus* (Sweet pea)
   l. *Lathyrus sativus* (Grass pea)
   m. *Lathyrus tingitanus* (Tangier pea)
   n. *Lathyrus latiofolius* (Perennial sweet pea)
   o. *Lathyrus ochrus* (Ochrus)
   p. *Lathyrus silvestris* (Flat pea)
   q. *Pisum sativum*
   r. *Pisum hortense* (Garden pea)
   s. *Pisum sativa arvense* (Canada field pea)
   t. *Pisum arvense* var. (Austrian winter pea)

I. Tribe *Phaseoleae*
  1. Subtribe *Glycininae*
   a. *Amphicarpa (monoica)* (Hog peanut)
   b. *Glycine hispida* (Soybean)
   c. *Clitoria mariana*
   d. *Centrosema virginianum*
  2. Subtribe *Erythrininae*
   a. *Apios tuberosa* (Ground nut)
   b. *Erythrina indica*
  3. Subtribe *Galactiinae*
   a. *Galactia regularis* (Milk pea)
  4. Subtribe *Diocleinae*
   a. *Pueraria thunbergibiana* (Kudzu)
   b. *Canavalia eusiformis* (Jackbean)
  5. Subtribe *Cajaninae*
   a. *Rhynchosia latifolia* (Prairie rhyncosia)

6. Subtribe *Phaseolinae*
   a. *Phaseolus acutifolius*
   b. *Phaseolus polystachys* (Wild bean)
   c. *Phaseolus vulgaris* (Kidney or string bean)
   d. *Phaseolus lunatus* (limensis) (Lima bean)
   e. *Phaseolus multiflorus* (Scarlet runner)
   f. *Phaseolus angularis* (Adzuki bean)
   g. *Phaseolus acontifolius* (Moth bean)
   h. *Phaseolus aureus* (Mung bean)
   i. *Phaseolus mungo* (Urd)
   j. *Phaseolus vulgaris* var. (Field bean, Navy bean)
   k. *Phaseolus* sp.
   l. *Strophostyles helvola* (Trailing wild bean)
   m. *Strophostyles pauciflora* (Small wild bean)
   n. *Vigna sinensis* (Cow pea)
   o. *Dolichos lablab* (Egyptian or black bean)
   p. *Stizolobium deeringianum* (Florida velvet bean)

From a study of this outline it is obvious that relatively few of the vast number of legume species are included. An attempt was made, however, to list the more important cultivated and wild species which are readily available for study. Wilson and Sarles (7) have published a very complete list of all the legumes from the nodules of which the root nodule bacteria have been isolated and studied.

HOW THE CULTURES OF RHIZOBIA ARE IDENTIFIED

Assume that a pure culture of *Rhizobium* has been isolated from a red clover nodule. In the outline, red clover, *Trifolium pratense*, is found under 3 Subfamily *Papilionatae*, D Tribe *Trifolieae*, O no Subtribe, k *Trifolium pratense*. Its number would be 3DOk1; the figure 1 added after 3DOk would represent the first isolation or the first red clover culture to be numbered. A second isolation or the second culture to be numbered would be 3DOk2. If more than one colony is picked from a plate poured from the first nodule isolation, the second and third culture obtained in this manner would be numbered 3DOk1(a) and 3DOk1(b), respectively. Several other examples are given here to show how the scheme works. An isolation is made and a pure culture obtained from a Korean lespedeza nodule. Its designation would be 3G5i1, from 3 Subfamily, G Tribe *Hedysareae*, 5 Subtribe *Desmodiinae*, and i *Lespedeza stipulacea*. A pure culture of soybean bacteria would be 3I1b1, from 3 Subfamily, I Tribe *Phaseoleae*, 1 Subtribe *Glycininae*, and b *Glycine hispida*.

A record is kept on filing cards for each culture showing its identification number, name of the organism, scientific and common name of the host plant, source of nodule, date of isolation, growth characteristics on different media, nitrogen-fixation data and microscopic examination. A sample card for an alfalfa culture is shown below. This additional record contains the more specific data for reference when necessary.

Laboratory No. ....
3DOa2

Name of Organism: ....
Rhizobium meliloti

Host plant: Scientific Name ....
Medicago sativa

Common Name ....
Alfalfa (Grimm)

Source of Nodules ....
Waukesha Co. Wisconsin

Date of Isolation ....
September 1938

Test On:

No. 79 ....
Whitish, flaky medium heavy growth

L.M. ....
Acid serum zone .

A.M. ....
Medium growth white smooth

Ca G.P. ....

B.T.B. 79 ....
Acid

Host Plant ....
Alfalfa 57 mg. N. 20 plants 6-13-40

Microscopic Examination ....
O.K.

DISCUSSION

This scheme of numbering cultures of *Rhizobia* has been in successful operation in our laboratory for a number of years and has aroused considerable interest among other workers. The suggestion has been made that the use of Roman numerals be used to affiliate each culture with its proper cross-inoculation group. This might be desirable for those workers who do not work with *Rhizobia* exclusively. Fred, Baldwin and McCoy (4) have listed the various cross-inoculation groups designating each group by a Roman numeral, and these could easily be affixed to each culture number for this identification.

Some of the advantages of this identification scheme for numbering cultures of *Rhizobia* may be summarized as follows:

1. It would standardize the labeling of all legume cultures.

2. It immediately identifies the culture with respect to host plant source history and cross-inoculation group affiliation.

3. The increasing numbers of cultures isolated from any species of legume does not necessitate changing existing culture numbers regardless of how many may accumulate.

4. The identification number of any *Rhizobia* culture will be much shorter than actually writing the name of the legume and using a simple number afterward, such as Crimson clover 1, 2, etc., or Soybean 1, 2, etc.

5. The last figure in each culture number furnishes a ready chronological reference as to the approximate date of isolation. Exact dates are kept on the cards previously described.

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FACTORS INFLUENCING THE SOLUBILITY OF IRON AND PHOSPHORUS IN CHLOROTIC AND NONCHLOROTIC AREAS OF HYRUM CLAY LOAM

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The soils of Utah and adjoining states are predominantly calcareous, and the pH is generally higher than considered optimum for most crops. Consequently, fertility problems of this area differ from those of humid regions. As in humid sections, phosphate unavailability is a problem, but the phosphate compounds in arid soils appear to be different from those in humid soils. Some phosphate fertilizers which are successful in humid areas bring about little plant response in the Rocky Mountain region. Probably the most extensive study of phosphate relations in calcareous soils has been made by McGeorge and associates in Arizona. According to their findings, the principal factors influencing phosphate availability under Arizona conditions are pH, soluble salts, drainage, aeration conditions, and organic matter content (9).

Another common problem associated with western calcareous soils is unavailability of iron and manganese, causing chlorosis. Chlorosis resulting from lack of iron is more marked in Utah than that resulting from manganese deficiency. In this state chlorosis attacks especially fruit trees, berries, roses, and ornamental shrubs. It occurs sporadically, and in some affected orchards badly·chlorotic trees may be surrounded by healthy trees of the same variety.

Many studies have attempted to characterize soils on which chlorosis is prevalent. In general no relation has been found between chlorosis and the total iron content or the pH of soils. Ciferri (4) found greater quantities of lime in chlorotic soils than in closely adjacent nonchlorotic soils. Menchikousky and Preffeles (11) found that in the Jordan Valley chlorosis occurred on soils high in chlorides. Lipman (6) reported that in California chlorosis is associated with relatively high soil contents of phosphorus, potassium, and sodium, and with low total soluble salts, nitrates, calcium, and magnesium, as compared with normal soils.

No well-developed methods have been presented for estimating available iron in calcareous soils. Monnier and Kuczraski (12) demonstrated that in a calcareous soil, traces of iron could be brought into solution by leaching with solutions of acetic, oxalic, citric, and tartaric acids. Rigg (13) found that in New Zealand soils, iron soluble in 0.1 to 5 per cent oxalic acid was much lower in areas where animals suffered from bush sickness than in areas where animals were normal.
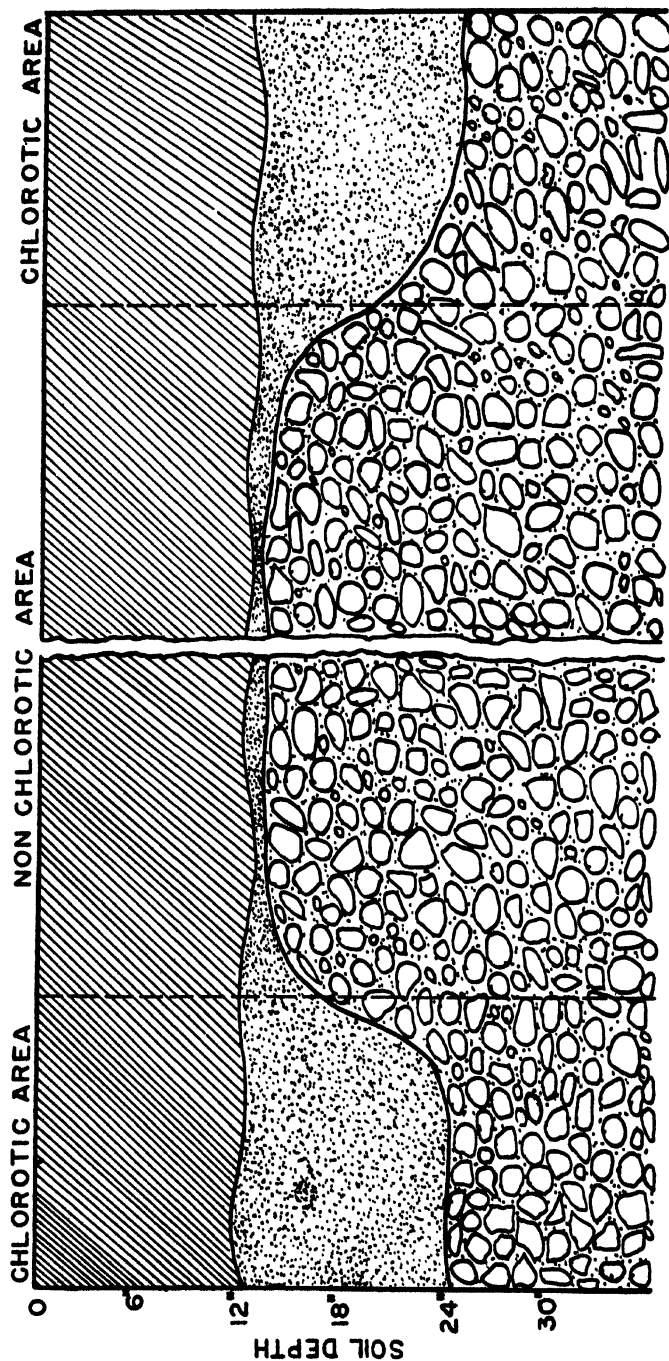
SKETCH SHOWING CROSS SECTION OF SOIL PROFILE CHARACTERISTICS OF ADJACENT CHLOROTIC AND NON-CHLOROTIC AREAS IN THE COLLEGE GRAPE TESTING PLOT. (HYRUM CLAY LOAM SOIL.)

INDICATES FRIABLE CLAY LOAM.
INDICATES COMPACT FINE SANDY CLAY TO FINE SANDY LOAM.
INDICATES COARSE GRAVEL AND COBBLESTONES

Fig. 1.

OUTLINE OF PROCEDURE

The present study was planned to investigate the interrelations of a number of characteristics of a highly calcareous soil, particularly in relation to the occurrence of chlorosis.

A survey of Utah County, Utah, indicated that about 85 per cent of chlorosis occurs on the Pleasant Grove, Orem, and Mapleton soils series. These soils are closely similar in profile characteristics to the Hyrum series which has a much wider distribution.

For several years a small plot of land on the Utah State Agricultural College campus, in an area of Hyrum clay loam, has been used as a testing plot to determine the susceptibility of plants to chlorosis. Concord grapes planted on the land became severely chlorotic except in a small area in the south central part of the field, where plants remained green and healthy. Injections and spraying with iron solutions have shown the chlorotic plants to be suffering from lack of available iron. (For convenience, soils producing chlorotic plants will be termed "chlorotic" soils, whereas those producing normally green plants will be termed "nonchlorotic" soils.)

A sewer pipe trench recently dug across the areas of chlorotic and nonchlorotic soils in the testing plot revealed a distinct difference in soil profile characteristics between the two areas. A sketch of the soil profile cutting across the field through the areas of chlorotic and nonchlorotic soils is shown in Figure 1.

This soil is very stratified, having been laid down as a delta by Logan River at the time Lake Bonneville covered much of northern and central Utah. The top soil of the field is about 12 inches deep and is a highly calcareous, dark brown, friable clay loam. It has a uniform appearance throughout the field. The top soil grades abruptly into a compact light gray layer varying in texture from a sandy clay at the top to a fine sand at greater depths. The depth of this second layer seems to be the principal profile difference between the chlorotic and nonchlorotic areas of soil in the field. The layer is much deeper in the chlorotic areas. The compact second layer grades into a third layer of coarse sand, gravel, and cobblestones. Near the top of the third layer is some lime accumulation on the lower sides of the cobblestones.

The initial soil samples were taken with an auger and shovel. Eight locations, four of chlorotic soil and four of nonchlorotic, were sampled at 6-inch intervals to depths of 3 feet. Later, when the trench was dug across the field, additional soil samples were taken from the first, second, third, and fourth feet of the soil at four points in the chlorotic area and at four points in closely adjacent nonchlorotic areas.

Unless otherwise specified, analyses are based on that fraction of soil passing a 20-mesh sieve. All unnecessary grinding was avoided because in some instances grinding greatly affects the determinations. This is somewhat in agreement with the statement of Chapman (3) that under

irrigation conditions soil particles frequently become coated with lime, making plant nutrients less available.

The pH values of the soil were determined with a Beckman glass electrode pH meter. Determinations were made with a soil paste (two parts soil, one part water) and with a 1:10 dilution. The determinations were repeated with tap water and with boiled distilled water. Total inorganic carbonates were determined by the method of Schollenberger (14).

Carbon dioxide-soluble calcium was determined by bubbling carbon dioxide through a 1:20 soil-distilled water suspension for 30 minutes, filtering, and determining calcium by the soap titration method. (1). Various lengths of time and rates of flow for passing the carbon dioxide through the soil suspension showed that reproducible results could be obtained with the conditions selected.

Available phosphorus was extracted by the pH 5.0 acetate buffer proposed by Dahlberg and Brown (5). This type of extraction has been found to correlate better with crop responses in Utah soils than other methods investigated.

Oxalic acid-soluble iron was determined by shaking the soil in a 1:5 dilution with the acid solution for 10 minutes, allowing to settle for 30 minutes, filtering, adding 5 ml. of 1-1 hydrochloric acid to 25 ml. of filtrate, oxidizing the oxalate with potassium permanganate, and developing color by the addition of potassium thiocyanate solution. Color intensity readings were taken with an Aminco Type F photo-electric colorimeter.

RESULTS

A general view of the textural characteristics of the soil profiles is shown by the percentage of soil, from each successive foot in depth, which could pass a 20-mesh sieve without having the larger sand and gravel particles broken up. The averages of these data for the chlorotic and nonchlorotic soils are shown in Table 1. These data are included in the statistical analyses as the only usable measurements indicating soil depth, texture, aeration, and drainage.

The data in Table 1 support the profile sketch of Figure 1. In the nonchlorotic soil, a predominance of particles larger than the holes of a

TABLE 1

AVERAGE PERCENTAGE OF CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM
SOIL PASSING THROUGH A TWENTY-MESH SIEVE

DEPTH OF SOIL	SOIL PASSING THROUGH A 20-MESH SIEVE	
	CHLOROTIC SOIL	NONCHLOROTIC SOIL
	Percentage	*Percentage*
0-12"	97.2	95.1
12-24"	98.0	26.8
24-36"	99.0	15.3
36-48"	56.6	30.8

20-mesh screen occur in the second foot while in the chlorotic soil such a condition is not encountered until the fourth foot.

A 1:10 water extract of the soil samples was made, and the soluble calcium, magnesium, sodium, potassium, chlorides, sulfates, bicarbonates, nitrates, and phosphates in the extracts were determined. No consistent difference between samples or between chlorotic and nonchlorotic soils was found, and so the data are not reported.

With tap water the pH data were closely similar in the 2:1 and 1:10 soil-water mixtures. The data obtained with the 2:1 ratio with boiled distilled water were only slightly different from the determinations with tap water. The 1:10 dilution with boiled distilled water, however, gave much higher values. Since such a figure is described by McGeorge (7, 8) as representing the maximum pH brought about by the hydrolysis of basic soil constituents, it is termed "hydrolyzable" pH. The average pH data for the 2:1 ratio of soil and tap water and the 1:10 ratio of soil and boiled distilled water (hydrolyzable pH) are shown in Table 2.

TABLE 2

AVERAGE pH VALUES FOR CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL IN 2:1 RATIO WITH TAP WATER AND 1:10 RATIO WITH BOILED DISTILLED WATER

DEPTH OF SAMPLE	CHLOROTIC SOIL		NONCHLOROTIC SOIL	
	pH in Tap Water	Hydrolyzable pH	pH in Tap Water	Hydrolyzable pH
0-12"	8.19	8.81	8.31	8.57
12-24"	8.25	9.18	8.41	8.93
24-36"	8.33	9.25	8.39	9.31
36-48"	8.29	9.34	8.44	9.38

There was no significant difference in the pH of chlorotic and non-chlorotic soils moistened with tap water. The chlorotic soils did, however, have a significantly higher hydrolyzable pH. The difference was greater in the 0-12-inch and 12-24-inch depths than at lower depths.

The data for total inorganic carbonates and carbon dioxide-soluble calcium are presented in Table 3. The average values for both determinations are higher in the chlorotic soils at all depths. In the case of total

TABLE 3

AVERAGE PERCENTAGE TOTAL CALCIUM CARBONATE AND PERCENTAGE CARBON DIOXIDE-SOLUBLE CALCIUM IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM

DEPTH OF SAMPLE	CHLOROTIC SOIL		NONCHLOROTIC SOIL	
	Total $CaCO_3$	CO_2-soluble Ca	Total $CaCO_3$	CO_2-soluble Ca
	Percentage	Percentage	Percentage	Percentage
0-12"	22.7	0.481	5.20	0.208
12-24"	47.4	0.553	39.20	0.203
24-36"	53.4	0.534	47.40	0.381
36-48"	50.6	0.438	46.30	0.294

carbonates, however, the differences are only consistent in the 0-12, and 12-24-inch depths.

The carbon dioxide-soluble calcium and the percentage of total lime expressed as calcium carbonate in several samples of native limestone formations which serve as parent materials for many Utah soils are shown in Table 4. The samples were ground to pass a 40-mesh screen. The carbon dioxide-soluble calcium in the limestone formations was similar in all cases except for the marl which was definitely more soluble.

TABLE 4
TOTAL LIME AND CARBON DIOXIDE-SOLUBLE CALCIUM IN VARIOUS COMMON LIMESTONES OF UTAH

Type of Limestone	Source	Total $CaCO_3$	CO_2-soluble Ca
		Percentage	Percentage
White Marl (Pleistocene Bonneville)....	White Valley, Utah	59.6	0.816
Limestone	Cotton Wood Canyon	42.0	0.68
Fresh water algal Limestone	Thistle Canyon	37 9	0.718
Limestone, Salt Lake formation	Collinston area	31.0	0.696
Limestone, Madison formation	Providence Canyon	38.2	0.673
Limestone	San Juan County	42.8	0.696

Several solvents were tried in an attempt to distinguish between the solubility of iron in the chlorotic and nonchlorotic soil samples. Some differences were obtained with acetic, tartaric, and citric acids indicating a generally greater solubility of iron in the nonchlorotic soils, but much greater differences were obtained with oxalic acid. The differences obtained with oxalic acid, however, were somewhat dependent upon the proportion of acid to soil. With a 1:5 suspension, a strength of less than about 0.25 per cent usually extracted only traces of iron, while solutions more concentrated than 1.5 per cent extracted large quantities of iron. Differences between the chlorotic conditions of the soil were not distinct, however, with the more concentrated solutions of oxalic acid. Best results with this soil were obtained with 0.25 and 0.5 per cent oxalic acid. The data for these two strengths are reported in Table 5.

TABLE 5
AVERAGE OXALIC ACID-SOLUBLE IRON IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL

DEPTH OF SAMPLE	SOLUBLE IRON IN CHLOROTIC SOIL		SOLUBLE IRON IN NONCHLOROTIC SOIL	
	In 0 25% Oxalic Acid	In 0 5% Oxalic Acid	In 0 25% Oxalic Acid	In 0 5% Oxalic Acid
	ppm	ppm	ppm	ppm
0-12"	0.85	2.05	3.00	19.08
12-24"	0.45	1.01	2.33	22.50
24-36"	0.60	0.70	0.20	1.45
36-48"	1.03	1.15	0.44	8.96

The principal differences in iron solubility occurred in the upper 24 inches. Since most plants have a larger proportion of feeder roots concentrated above the 24-inch depth, the data give a clear distinction between the chlorotic and nonchlorotic soils.

To check the influence on some of the determinations of fineness of grinding the soil, some samples were divided into portions which were ground to pass through 20, 40, 60, 80, and 100-mesh sieves. Iron soluble in 0.5 per cent oxalic acid and carbon dioxide-soluble calcium in portions of one sample ground to the different degrees of fineness are shown in Figure 2. The data indicate that the grinding procedure increases carbon dioxide-soluble calcium relatively more than soluble iron.
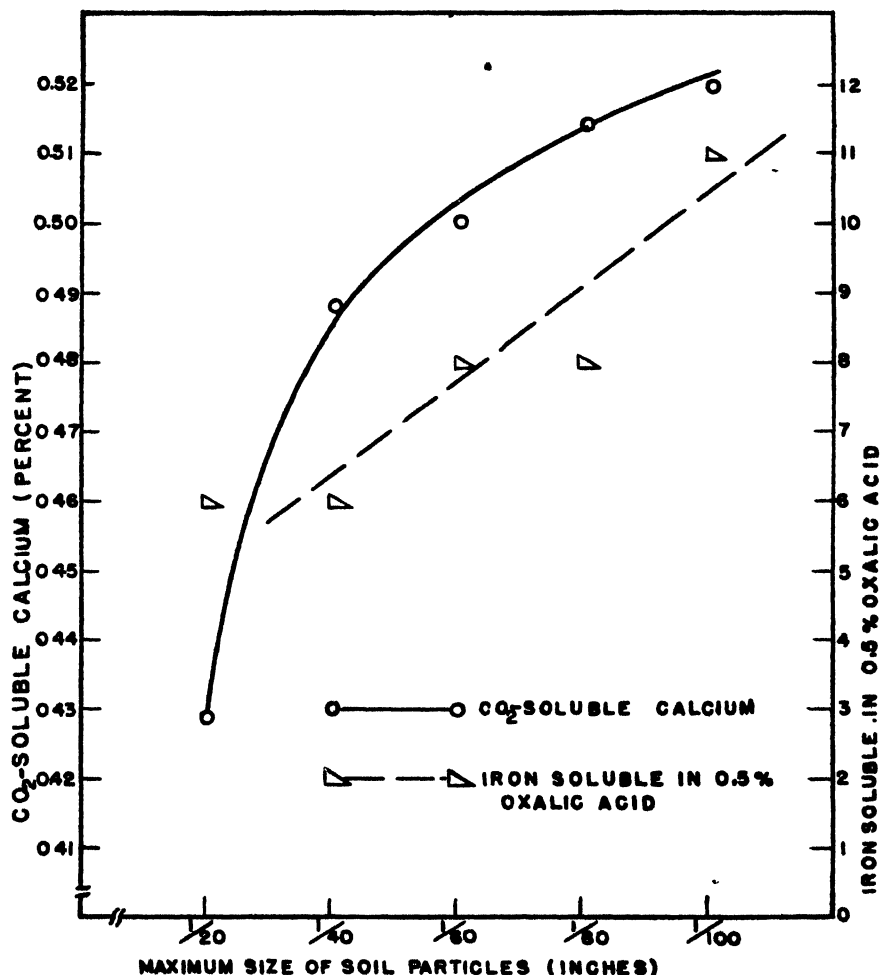
Fig. 2.—Influence of fineness of grinding of soil on carbon dioxide-soluble calcium and iron soluble in 0.5 per cent oxalic acid.

Since phosphate unavailability is also common in highly calcareous soils, and since its concentration has been reported related to the occurrence of chlorosis, phosphate determinations were included in the analyses. Averages of the results obtained are given in Table 6. Although average available phosphorus is slightly higher in the nonchlorotic soil, the differences are not significant.

TABLE 6

AVERAGE AVAILABLE PHOSPHORUS IN CHLOROTIC AND NONCHLOROTIC HYRUM CLAY LOAM SOIL

DEPTH OF SAMPLE	AVAILABLE PHOSPHORUS	
	Chlorotic Soil	Nonchlorotic Soil
	ppm	ppm
0-12″	2.01	2.33
12-24″	0.55	0.99
24-36″	0.09	1.18
36-48″	0.065	0.55

Simple correlation coefficients between the data for the various determinations were calculated (Table 7). From these calculations, the following relations are indicated:

TABLE 7

CORRELATION COEFFICIENTS BETWEEN VARIOUS VALUES FOR HYRUM CLAY LOAM SOIL

	Fe soluble in 0 5% Oxalic Acid	Available Phosphorus	Percentage Soil Passing 20-mesh Sieve	Percentage CaCO₃	CO₂-Soluble Ca	Hydrolyzable pH	pH in Tap Water
Fe soluble in 0.5% oxalic acid..	......	0.264	−0.245	−0.506	−0.705	−0.416	−0.099
Available phosphorus	0.264	......	0.132	−0.869	−0.917	0.776	−0.202
Percentage soil passing 20-mesh sieve	−0.245	0.132	......	−0.288	0.399	−0.376	−0.520
Percentage total CaCO₃	−0.506	−0.869	−0.288	......	0.582	0.866	0.401
CO₂-soluble Ca ..	−0.705	−0.917	0.399	0.582	......	0.477	−0.227
Hydrolyzable pH.	−0.416	0.776	−0.376	0.866	0.477	......	0.300
pH in tap water .	−0.099	−0.202	−0.520	0.401	−0.227	0.300	......

Least significant value equals 0.367
Least highly significant value equals 0.470

1. Soil pH with tap water was significantly correlated only with percentage lime. It had a highly significant negative correlation with the percentage of soil passing a 20-mesh screen. The latter relation appears owing to the tendency for pH to increase with soil depth.

2. Hydrolyzable pH had a highly significant correlation with total lime, carbon dioxide-soluble calcium, and available phosphorus. It had a significantly negative correlation with soluble iron and percentage of soil

passing a 20-mesh screen. The correlation coefficient between hydrolyzable pH and pH in tap water was just below the level of significance.

3. Percentage of total calcium carbonate had a highly significant correlation with all other determinations except percentage of soil passing a 20-mesh screen. The correlation with soluble iron was negative.

4. Of all factors investigated, carbon dioxide-soluble calcium showed the closest relation to soluble iron and available phosphorus. It also had a highly significant correlation with total lime and hydrolyzable pH, but was not significantly related to pH in tap water.

5. Oxalic acid-soluble iron was most closely related to carbon dioxide-soluble calcium and total calcium carbonate. The relationship between soluble iron and available phosphorus did not reach the level of significance.

6. Available phosphorus was highly correlated with total calcium carbonate, carbon dioxide-soluble calcium, and hydrolyzable pH.

On the basis of the close relations between oxalic acid-soluble iron, total calcium carbonate, and carbon dioxide-soluble calcium, a multiple correlation was calculated. The "R" value for this calculation was 0.714 which gave only a very slight decrease in the standard error of estimate for soluble iron over the use of the simple regression equation using carbon dioxide-soluble calcium alone for the estimation. An evaluation of the "β" values for this calculation showed that 80 per cent of the accounted-for variation in soluble iron could be based on carbon dioxide-soluble calcium and only 19 per cent on percentage of total calcium carbonate in the soil.

A similar calculation for the estimate of hydrolyzable pH based on total carbonates and carbon dioxide-soluble calcium indicated that carbon dioxide-soluble calcium had no value when used in addition to total lime for estimating hydrolyzable pH.

The multiple correlation coefficient between available phosphorus, total calcium carbonate, and carbon dioxide-soluble calcium was found to be 0.972. This represents a standard error of estimate for available phosphorus of 0.22 compared with 0.36 using carbon dioxide-soluble calcium alone and 0.45 using total calcium carbonate. Evaluation of the "β" values indicated that about 52 per cent of the accounted-for variation could be based on carbon dioxide-soluble calcium and 48 per cent on total calcium carbonate.

DISCUSSION

The results obtained in this investigation are not considered particularly representative of conditions in other calcareous or chlorotic soils. Soil conditions associated with chlorosis and phosphate unavailability are apparently so variable and complex that an introductory survey of many soils would probably lead to few specific conclusions. In some soils chlorosis may result largely from poor drainage, in other cases from alkali, but in many instances the only conditions noted to be associated with

chlorosis are a moderately high pH (usually 8.0 or above in Utah) and the presence of lime.

Accordingly, an intensive study of one soil producing chlorotic and green plants in well-outlined adjacent areas was made to determine some of the soil conditions closely associated with the disease. It is hoped that the results obtained with this one soil will be helpful in the study of iron and phosphate availability in other Utah soils.

The pH data are of interest in that pH of the soil moistened with tap water was not closely related with other soil characteristics investigated, while the maximum pH developed in a 1:10 soil suspension with boiled distilled water was significantly related to most of the factors. McGeorge (8) has pointed out that pH with tap water is close to the value existing under irrigated conditions, whereas hydrolyzable pH is much higher than exists under field conditions. The present study seems to indicate, however, that hydrolyzable pH may be more closely related to soil reactions than had been previously indicated.

The higher content of lime in the upper horizons of chlorotic soil compared with nearby nonchlorotic soil is in agreement with the findings of Ciferri (4). Since the soil studied was laid down by water and the topsoil has a similar depth and appearance over the entire field, the present differences in lime content are apparently owing to differences in internal drainage. Where the topsoil is closely underlain by coarse gravel, much of the lime has been leached, but where the compact sandy clay deposit underlies the topsoil, leaching has been retarded and the resulting soil is higher in lime and is chlorotic.

Except in such limited areas as that covered by the present study, it has not been possible to make any general distinction between chlorotic and nonchlorotic soils on the basis of percentage of total calcium carbonate alone. Chapman (3) has suggested that iron availability may be limited by the mobility of lime. He suggests that under irrigated conditions, lime coatings may be deposited around soil particles, thus limiting the nutrients available to many plants.

Attempts to distinguish a lime coating around soil particles by staining techniques were unsuccessful. Following these attempts, methods were sought to measure the mobility or solubility of lime in soil. Water-soluble calcium was determined on all samples, but the results showed no appreciable differences. Reaction of soil calcium with various oxalate solutions was also tried, and some differences were noted. This method is being further investigated.

The solubility of soil lime in carbon dioxide is an entirely arbitrary method for measuring mobility and gives somewhat different results under different conditions. Calcium carbonate may be brought entirely into solution upon prolonged agitation with water and a stream of carbon dioxide (Mellor (10)). Although the concentrations of carbon dioxide employed were much higher than occur in normal soils, the difference between the field and laboratory conditions is probably one of degree

rather than nature. Most studies on calcium carbonate indicate that there is only one predominating form in nature, and that under similar conditions the same reactions might be expected. The associations of calcium carbonate with other soil constituents appear very complex, however, and the relative activity is greatly altered by these relationships. This is indicated by the comparatively low pH of even highly calcareous soils compared with pure calcium carbonate or even limestones in similar states of division (Buehrer and Williams (2)).

Several methods for the measurement of iron solubility in soils were studied, but most of them were unsatisfactory. Soil extracts with distilled water or with water and carbon dioxide contained insufficient iron to give a test by the thiocyanate method. Extracts with 1 per cent solutions of citric and tartaric acid removed small amounts of iron from several of the soils, but the results were not as consistent as with oxalic acid. There is no evidence that iron soluble in 0.25 and 0.5 per cent oxalic acid represents that available to plants. But with the soil studied, good agreement was found between the iron brought into solution and the occurrence of chlorosis. A number of other soils have been subjected to iron extraction with oxalic acid solutions. In most cases chlorotic soils can be distinguished from closely adjacent nonchlorotic soils by the quantity of iron brought into solution. In some cases, however, the strength of oxalic acid must be altered. In one instance a strength of 1.5 per cent oxalic acid was required before a test for iron was obtained.

Acetate (pH 5.0) soluble phosphorus and oxalic acid soluble iron were related to the same soil characteristics but to a different degree. Both were most closely related to carbon dioxide-soluble calcium. But while the percentage of total lime in the soil accounted for almost as much variation in available phosphorus as carbon dioxide-soluble calcium, in the case of soluble iron it accounted for less than one-fourth as much variation as carbon dioxide-soluble calcium. These relations would seem to indicate that, while the availability of iron and phosphorus in the soil studied are influenced by many of the same factors, there must be factors not included in the present study which have different effects upon these two elements.

SUMMARY

1. The relations of a number of soil characteristics to oxalic acid-soluble iron and acetate (pH 5) soluble phosphorus in chlorotic and non-chlorotic areas of Hyrum clay loam soil have been investigated.

2. Chlorotic areas of the soil were characterized by a layer of compact sandy clay to fine sand ranging in depth from 2 to 3 feet and situated between the 12-inch layer of topsoil and the cobblestones and gravel below. In nonchlorotic areas of soil, this layer was not so compact and was only a few inches thick.

3. Iron was much more soluble in 0.5 per cent oxalic acid in nonchlorotic areas of soil than in chlorotic areas.

4. The pH values of the soils moistened with tap water were not significantly different in the chlorotic and nonchlorotic areas, but the pH values of 1:10 soil-boiled distilled water suspensions were higher in samples from chlorotic areas.

5. The percentage of total carbonates was much higher in the 0-12 and 12-24-inch depths of soil in the chlorotic areas as compared with the nonchlorotic areas.

6. Calcium soluble in a 1:20 suspension of water aerated with carbon dioxide for 30 minutes was employed as a measure of lime activity. The values obtained were higher for chlorotic than for nonchlorotic soil areas.

7. No appreciable differences in available phosphorus were found between the chlorotic and nonchlorotic soils.

8. The principal factors influencing oxalic acid-soluble iron were found to be carbon dioxide-soluble calcium and total calcium carbonate.

9. The principal factors influencing available phosphorus were carbon dioxide-soluble calcium, total calcium carbonate, and pH of a 1:10 suspension of soil in boiled distilled water.

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NUMBERS OF MICRO-ORGANISMS IN RELATION TO AGGREGATE SIZE[1]

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The effect of organic matter in the formation of stable aggregates has recently been attributed to the binding action of soil micro-organisms. Waksman and Martin (6) and Martin and Waksman (2) found that micro-organisms caused a marked aggregation of soil particles, the extent of the effect varying with the nature of the organisms and with the nature of the organic matter added. Peele (5) states that "the mucus produced by bacteria was found to be an effective binding agent in the formation of water-stable granules." He also found that cultures of fungi were effective in producing water-stable aggregates. Recently Myers and McCalla (4) studied the relationship between bacterial numbers and aggregation in some prairie soils, and found that maximum aggregation usually occurred some time after maximum bacterial numbers. As a result of their studies they concluded that the metabolic products of bacterial action function as the cementing agents.

In addition, it has been noted that organic materials which decompose readily are more effective in producing aggregates than are materials which decompose slowly. It has also been noted by Metzger and Hide (1, 3) that the large aggregates have a higher content of organic matter than the small aggregates.

In view of these studies which emphasize the importance of micro-organisms in relation to soil aggregation it appeared desirable to determine if there is significant variation in numbers of bacteria and fungi in aggregates of various sizes.

METHODS

The soils used in this study were Gilpin silt loam, an upland soil derived from noncalcareous sandstone and shale; Elk silt loam, a terrace soil having some calcareous parent material; and Pope silt loam, a bottomland soil washed in from noncalcareous sandstones and shales.

Fresh soil samples representing each of the three types were taken from the field and passed through a 7-mm. screen while still moist. The samples were then air-dried and samples taken for aggregate analysis according to the method of Yoder (7). As soon as the separation of aggregates was completed the screens were removed from the water, allowed to drain, and air-dried overnight. The air-dry samples were then

used for plating for numbers of bacteria and fungi. The soil samples were pulverized in a mortar to break up the aggregates before making dilutions. Plates were made in triplicate using sodium caseinate agar for bacteria and acid peptone glucose agar for fungi.

EXPERIMENTAL RESULTS

The numbers of bacteria and fungi in the various aggregates are given in Table 1. Although the largest numbers of bacteria in all three soils were found in the aggregates between 1 and 2 mm., and in two of the soil types the largest numbers of fungi appear in the same fraction, variations within triplicate plates were in most cases greater than variations between the numbers for the various aggregates. Consequently, no significance is attached to these differences.

TABLE 1

NUMBERS OF BACTERIA AND FUNGI PER GRAM IN SOIL AGGREGATES

SIZE OF AGGREGATES MM.	Gilpin		Elk		Pope	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
	millions	thousands	millions	thousands	millions	thousands
5.0	...	...	...	..	5.6	27
5.0 –2.0	10.8	190	8.9	38	6.0	25
2.0 –1.0	18.8	232	21.0	44	7.2	26
1.0 –0.5	14.6	174	8.6	35	6.0	19
0.5 –0.25	13.0	219	10.7	25	5.2	22
0.25–0.1	11.5	75	18.6	30	5.5	45

Numbers of actinomyces on the bacterial plates were determined in all three soils. These were found to represent 25 to 30 per cent of the colonies from the Gilpin, 15 to 25 per cent from the Elk, and 12 to 20 per cent from the Pope, but there were no variations which could be correlated with size of aggregates. Determinations of numbers of gum-producing bacteria on the Elk soil showed about the same number on all plates regardless of size of aggregates. Actual counts were not made on the other two soils, but no marked differences were evident.

The possibility that the washing method used in separating aggregates may have removed micro-organisms was recognized. There was also the possibility that during the process of wetting and drying there might have been changes in numbers. Consequently, a second sample of the Pope soil was dry-screened and numbers of micro-organisms determined on the dry-screened sample. The Pope soil was selected because over two-thirds of the large aggregates by dry-screening were found to be stable when wet-screened. The results are given in Table 2, together with results from wet-screening.

In the dry-screened sample, maximum numbers of bacteria and fungi were found in the 0.5 to 0.25-mm. aggregates, with a rapid decrease in the smaller fraction. Variations in bacterial numbers between plates were

TABLE 2

EFFECT OF DRY vs. WET-SCREENING ON NUMBERS OF MICRO-ORGANISMS PER GRAM IN SOIL AGGREGATES (POPE SOIL)

SIZE OF AGGREGATES MM.	BACTERIA (millions)		FUNGI (thousands)	
	Dry-Screened	Wet-Screened	Dry-Screened	Wet-Screened
5.0	4.3	5.6	19	27
5.0 -2.0	5.7	6.0	11	25
2.0 -1.0	5.7	7.2	18	26
1.0 -0.5	6.2	6.0	18	19
0.5 -0.25	7.5	5.2	23	26
0.25-0.1	3.6	5.5	13	45

greater than variations between aggregates except in the 5.0 and the 0.25 to 0.1 fractions, both of which were consistently lower than the other aggregates. Variations in number of fungi on triplicate plates were so high that the differences noted cannot be considered significant.

The numbers of bacteria and fungi were generally higher in the wet-screened soil, but the differences were in most cases within the limits of error. Although results from one sample cannot be considered as positive proof, it does not appear that the washing process materially influenced the numbers of organisms in the various aggregates.

DISCUSSION AND SUMMARY

The lack of correlation between numbers of bacteria and fungi and size of aggregates gives added support to the conclusions of Myers and McCalla (4) that the effect of micro-organisms in stabilizing soil aggregates is due to their products rather than to the organisms themselves. It is recognized that the type of organisms may be a more important factor than numbers, but in these studies there appeared to be little difference in types of organisms on the plates from various-sized aggregates. The products of decomposition or the gum produced by certain bacteria may account for the effect of bacteria which has been noted.

The effect of fungi does not appear to be related to the amount of mycelium, hence can only be accounted for either by its location with respect to the aggregate or by the products of decomposition. Both spores and fragments of mycelium were counted, but even the early counts which represent largely growth from pieces of mycelium did not show any significant differences.

It is recognized that this study is only preliminary and that the methods used are open to criticism. Further studies in which various types and amounts of organic matter are added and various micro-organisms used should be made to establish the relationship of micro-organisms to soil aggregation.

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